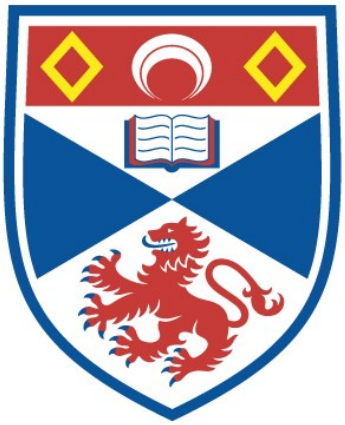
CHITOSE

Archibald Bradshaw Grant

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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C H I T O S E.

C H I T O S E

being a thesis

presented by

ARCHIBALD BRADSHAW GRANT

to the

UNIVERSITY of ST. ANDREWS

in application

for the degree

of

DOCTOR OF PHILOSOPHY.

Ms
1105

C E R T I F I C A T E.

I certify that Archibald Bradshaw Grant has spent eleven terms at research work under my direction, and that he has fulfilled the conditions of Ordinance No. 16, (St.Andrews,) and is qualified to submit the accompanying thesis for the degree of Ph.D.

St.Andrews. Director of Research.

C A R E E R.

I matriculated in the University of St. Andrews in October 1934 and followed the recognised course of study in the Faculty of Science for the degree of B.Sc.

In 1938 I passed the Second Science Degree examination in the subjects of Physiology and Chemistry and in June 1939 graduated B.Sc., with First Class Honours in Chemistry.

With the assistance of a Carnegie Research Scholarship throughout sessions 1939-40 and 1940-41 I carried out in the department of Physiological Chemistry research work which was interrupted by a period of service with H. M. Forces. During the session 1945-46 I was awarded a Rehabilitation Bursary by the New Zealand Government and resumed my research programme, the results of which form the subject of the present thesis.

ACKNOWLEDGEMENTS.

The writer here expresses his gratitude to Dr. A. Hynd for his kindly interest and valuable criticism throughout the course of this investigation.

Thanks are also tendered to the Carnegie Trust for the Universities of Scotland and to the New Zealand Government Department of Rehabilitation for the awards of Research Scholarships which have enabled the writer to undertake this work.

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PREVIOUS HISTORY OF CHITOSE.

In the hope of obtaining information regarding the structure and configuration of glucosamine, Ledderhose, in 1880 (Z. Physiol Chem., 4, 139), attempted to obtain one of the known hexoses by substituting the amino group of glucosamine by a hydroxyl group. On treating a solution of glucosamine hydrochloride with silver nitrite or potassium nitrite he found, by volumetric estimation, that the amount of nitrogen liberated during the reaction was in agreement with the following equation:-

$$C_6H_{11}O_5 \cdot NH_2 \cdot HCl + KNO_2 = C_6H_{12}O_6 + H_2O + KCl + N_2.$$

He isolated the resulting sugarlike compound as a stiff syrup which was readily soluble in absolute alcohol and from this solution it was precipitated by ether in the form of white amorphous flakes. Its aqueous solution reduced Fehling's solution, was dextrorotatory and was not fermented by yeast. He was unable to identify the material but considered that it was a normal hexose though not glucose.

These observations were confirmed by Tiemann (Ber. 17, 241) and Kueny (Z. Physiol Chem., 14, 330) but neither of these investigators was able to isolate from the syrup a crystalline product suitable for analysis, and it was considered that the process of deamination resulted in further decomposition beyond the formation of a hexose.

By mild oxidation of this syrup with bromine water Fischer and Tiemann (Ber. 27, 138) obtained an acid which they isolated in the form of its crystalline calcium salt. The compound was stable in air on heating up to 140°C. and lost no water of crystallisation. Analysis indicated the formula of the salt of a normal hexonic acid $(C_6H_{11}O_7)_2Ca$ but it differed in its properties from any of the known hexonic acids. Fischer and Tiemann gave the name Chitonic acid to this substance and Chitose to the product of glucosamine deamination from which it was derived.

They were able to oxidise glucosamine to the mono--carboxylic glucosaminic acid from which by deamination they expected to obtain the same chitonic acid. The material isolated however was not calcium chitonate but the crystalline calcium salt of a monocarboxylic acid which on drying in air lost four molecules of water of crystallisation. From the results of analysis the substance was given the formula $(C_6H_9O_6)_2Ca$. The acid was given the name Chitaric acid and was considered to be an anhydrohexonic acid $C_6H_{10}O_6$.

A small amount of glucosazone separated when Fischer and Tiemann subjected a solution of chitose to the action of phenylhydrazine and acetic acid.

In 1902, Neuberg, Wolff and Niemann (Ber. 35, 4009) by treatment of chitaric acid with Fenton's reagent, obtained a syrup which gave intense pentose reactions and

from this material they prepared d-arabinosazone. In view of this fact they were of the opinion that chitaric acid was a derivative of either d-arabinose or d-ribose and since in neither of the known arabinohexonic acids was there any tendency towards anhydride formation, they considered that chitaric acid was a ribohexonic acid or its anhydride.

They attempted to prepare crystalline chitose derivatives by deamination of a number of glucosamine hydrazones suggesting that the protection given to the aldehydic group in these cases might result in a purer deamination product but they were unsuccessful.

After prolonged treatment of chitose in a solution of methyl alcohol containing 1½% hydrochloric acid, they were able to isolate a crystalline methyl chitoside (mp. 169°C.). Examination of the compound showed that it contained one molecule of water of crystallisation and gave analysis in agreement with the formula of a normal methyl hexoside $C_7H_{14}O_6 . H_2O$.

Benzoylation of chitose by the Schotten-Baumann reaction resulted in the formation of a crystalline tribenzoyl derivative which they described as the tribenzoyl derivative of a hexose $C_{17}H_{24}O_9$.

By hydrolysis of the addition compound of chitose and hydrocyanic acid the same workers obtained an amorphous chitoheptonic acid from which they prepared a crystalline dibenzoyl derivative (mp. 117-120°C.), $C_{21}H_{22}O_{10}$.

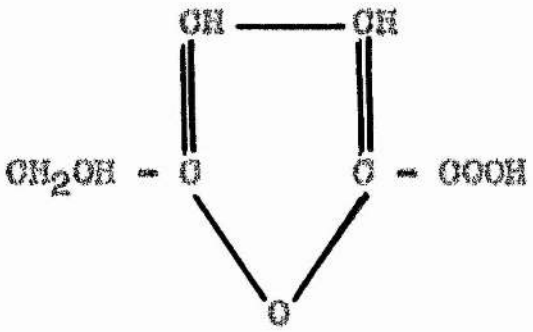
They prepared a non-crystalline chitose oxime and from its aqueous solution by addition of clear ammoniacal lead acetate an amorphous precipitate was obtained which gave analysis in agreement with the formula $(C_6H_{12}O_5:NOH)$--3PbO. This oxime was subjected to the process of Wohl's degradation but they were unable to identify the product which they considered to be a pentose and probably d-ribose.

As a result of this work Neuberg and his co-workers were of the opinion that chitose was a normal hexose with the probable d-ribohexose configuration.

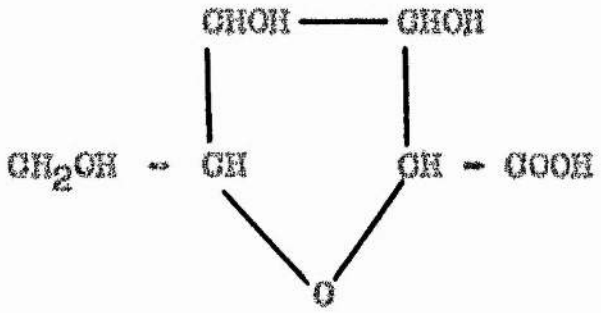
When it was pointed out that all of the eight theoretical hexonic acids were known and that the properties of chitonic acid corresponded with none of them Fischer and Andreae (Ber. 36, 2567) again examined the compound. They found that calcium chitonate in agreement with the previous observation analysed according to the formula $(C_6H_{11}O_7)_2$ Ca after drying in air at 140°C, but that when dried in vacuo at 140°C it slowly lost two molecules of water of crystallisation without undergoing further decomposition. They therefore ascribed to chitonic acid the formula of an anhydrohexonic acid. It seemed to them that chitonic and chitaric acids were stereoisomers and that since in neither case is the carboxyl group concerned in the anhydride formation, an ether linkage must exist within the molecule.

It was found that both acids, on treatment with acetic

anhydride in the presence of anhydrous sodium acetate were converted to the acetyl derivative of oxymethyl pyromucic acid previously discovered by von Hill and Jemminga (Am. 15, 181) who showed it to be ω-hydroxy-5-methylfuran-2--carboxyllic acid:-



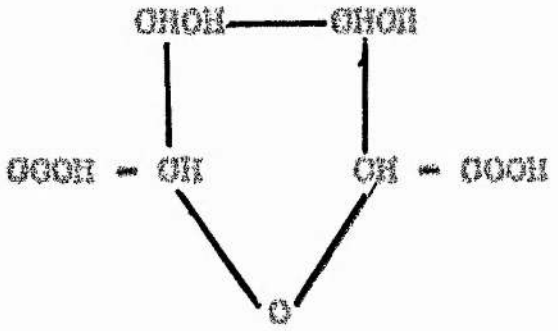
Accordingly chitonic and chitaric acids were regarded as stereoisomeric hydrofurane derivatives having the following formula:-



Fischer and Andreae expressed the opinion that the small quantities of glucosazone prepared from chitose were not derived from chitose itself but from glucosamine, present in the product as a result of incomplete deamination.

By oxidation of chitose with nitric acid Fischer and Tiemann (Ber. 27, 138) isolated isosaccharic acid, a compound

which had been previously prepared by oxidation of glucosamin hydrochloride (Tiemann, Ber. 17, 246), (Tiemann and Haarmann, Ber. 19, 1257). It was considered to be a dicarboxylic acid derivative of an anhydrohexose having the probable structure:



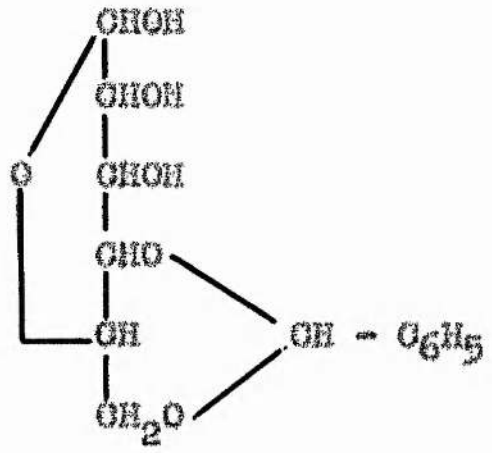
The compound and its diethyl ester were found to exhibit unusual properties, existing in hydrated forms from which were prepared both diacetyl and tetraacetyl derivatives (Tiemann and Haarmann, Ber. 19, 1257).

Irvine, McNicoll and Hynd, (J.C.S., 101, 250), by deamination of triacetylmethyl glucosamine hydrobromide isolated an unerystallisable product from which a number of acetyl groups had been split off. The substance strongly reduced Fehling's solution indicating the loss of the glucosidic methyl group but they were unable to identify it.

Irvine and Hynd (J.C.S., 101, 1128) prepared from glucosamine, dimethylaminomethylglucoside which was deaminated on treatment with barium hydroxide in aqueous solution and from the reaction mixture they obtained a

syrup having properties closely resembling those of α-methylglucoside. Methylation of this product resulted in what appeared to be tetramethyl methylglucoside which by hydrolysis was converted to tetramethyl glucose. The latter, hydrolysed with hydriodic acid, formed a syrupy product having constents in exact agreement with the standard values of d-glucose and gave crystalline d-glucosazone with phenylhydrazine.

These workers, in a later publication (J.C.S., 105, 698) described the deamination of the benzylidene derivative of aminomethylglucoside hydrochloride in alkaline solution and the resulting amorphous nitrogen free product. From analysis and examination of the properties of the latter it was given the following structure:-

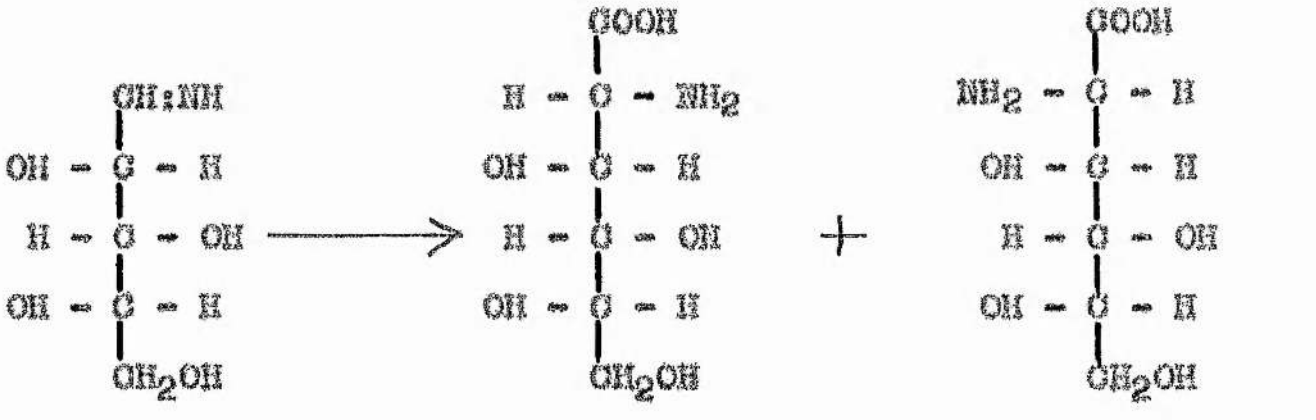


Removal of the benzaldehyde group by acid hydrolysis gave a product having the properties of d-mannose, and it was definitely identified as such by the preparation from it of the crystalline derivatives, mannose anilide and methyl

mannoside.

It was reported by Levene and LaForge (J. Biol. Chem. 20, 433) that oxidation of chitaric acid with nitric acid resulted in the formation of an isomer of isosaccharic acid which they called epiisosaccharic acid. They postulated that with either glucosamine or glucosaminic acid, a Walden inversion on carbon atom 2 accompanied the process of desamination and anhydride formation, whereas, in the other case, no inversion occurred. Consequently chitonic acid and chitaric acid are epimers, one being 2:5anhydrogluconic acid and the other 2:5anhydromannonic acid. Similarly isosaccharic and epiisosaccharic acids are the epimeric 2:5anhydrosaccharic acid and 2:5 anhydromannosaccharic acid.

In order to clear up the configurational uncertainty with regard to these acids Levene and LaForge (J. Biol. Chem. 21, 351) undertook a synthesis outlined as follows:-



Xyloseimine Idosaminic acid Gulosaminic acid

Only one of the two possible isomers was isolated from the

reaction mixture and it was uncertain whether this product was idosaminic acid or gulosaminic acid. On deamination of the compound and oxidation of the product a 2:5anhydro-hexose dicarboxylic acid should be obtained. From the above structure it can be seen that on such treatment one of these two hexosaminic acids would give rise to 2:5anhydrosaccharic acid while the other would produce 2:5 anhydroidosaccharic acid irrespective of whether or not Walden inversion on carbon atom 2 is involved in the deamination of a hexosaminic acid. The anhydro acid actually obtained from the xylohexo-saminic acid differed in its properties from both isosaccharic acid and epiisosaccharic acid and was accordingly given the configuration of 2:5anhydroidosaccharic acid.

Since Fischer and his co-workers had shown that as a general rule, deamination of amino acids and their esters yielded stereoisomers and not identical hydroxyacids Levene and LaForge considered that 2:5anhydrosaccharic acid might be obtained by deamination of the lactone (inner ester) of their xylohexosaminic acid and subsequent oxidation. This they found to be the case and the acid obtained was identical with epiisosaccharic acid. Accordingly the configuration of epiisosaccharic acid must be that of 2:5anhydrosaccharic acid and therefore isosaccharic acid is 2:5anhydromanno--saccharic acid. Consequently chitose is 2:5anhydromannose, chitonic acid is 2:5anhydromannonic acid and chitaric acid is 2:5anhydrogluconic acid.

By treatment of glucosaminic acid with pyridine Levene (*J.* Biol. Chem. 36, 73) prepared epiglucosaminic acid which decomposed on deamination to form chitonic acid. Unlike the xylohexosaminic acid which he had previously synthesised both epiglucosaminic acid and its lactone on deamination gave rise to chitonic acid instead of two epimers. From epiglucosaminic acid he prepared epiglucosamine which, on deamination and oxidation, formed saccharic acid and not epiisosaccharic acid. He was unable to oxidise epiglucosamine back to epiglucosaminic acid but, by mild oxidation with mercuric oxide, he obtained crystalline epichitose, the epimer of chitose, having the 2:5anhydroglucose configuration.

Nobuyoski Suzuki (*J.* Biol. Chem. 38, 1) reported that, after feeding chitose to rabbits, he isolated from the urine ω-hydroxy-5-methylfuren-2-carboxylic acid (hydroxymethyl pyromucic acid), previously prepared by Fischer by treatment of chitonic and chiteric acids with acetic anhydride.

Ambrecht (Biochem. Zeits. 95, 108) found that chitosan on treatment with nitrous acid was completely deaminated and hydrolysed and from the reaction mixture isolated a syrup which, he claimed, was identical with chitose. He prepared from this an osazone (mp. 202°C) which he claimed to be distinct from glucosazone and which analysed according

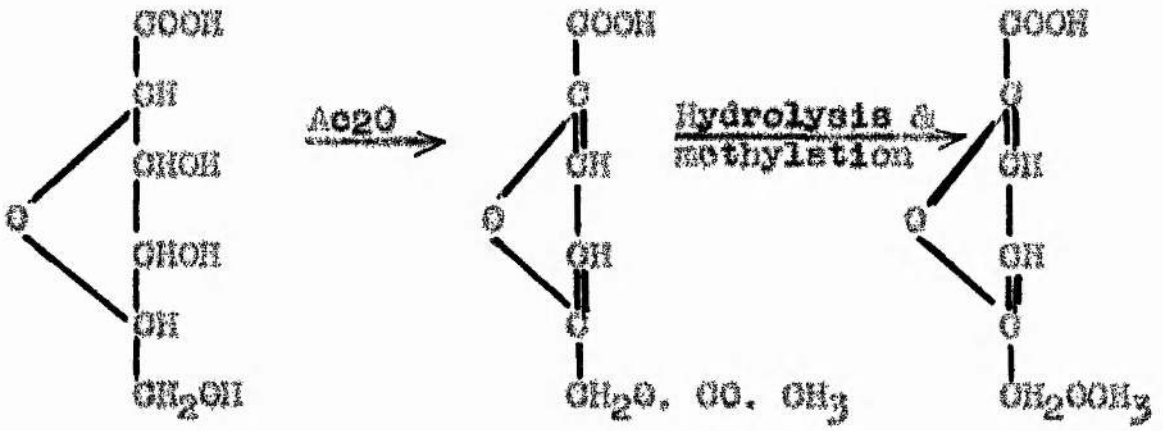
to the formula of a normal hexosazone. His osazone differed from glucosazone in its rotatory power and solubility. In view of this fact he considered it necessary to investigate Tiemann's statement that glucosazone is obtained from the deamination product of glucosamine hydrochloride. He therefore repeated Tiemann's experiment and the osazone he isolated was identical with that from deaminated chitosan. On mixing the osazones of chitose from the deamination of both glucosamine and chitosan, there was no depression of melting point but, on mixing either with glucosazone, a depression of melting point was observed. He claimed that the process of deamination in both cases is one of a very complicated nature and that chitose syrup is a mixture of many different substances. He retained the notation chitose for that one constituent which gave rise to his osazone. By oxidation of chitose with nitric acid he obtained an acid which, with cinchonine, gave a crystalline salt (mp. 200°C.) analysing in agreement with the cinchonine salt of an anhydrohexonic acid and not of a dicarboxylic acid as was expected. Ambrecht supported the opinion of Neuberg that chitose was a genuine hexose of the formula $C_6H_{12}O_6$.

Levene (J. Biol. Chem., 52, 135) has examined the optical behaviour of solutions of epichitose, chitonic acid and chiteric acid. It was found that epichitose solutions do not exhibit the phenomenon of mutarotation.

This together with the fact that it shows a markedly greater reducing power towards solutions of potassium permanganate than ordinary sugars, supports his conclusion that epichitose can only have an aldehydic structure. This fact is also borne out by examination of a molecular model constructed according to the glucose configuration, from which it was apparent that the hydroxyl group on carbon atom 4 is situated in the trans position from carbon atom 1 with respect to the plane formed by the 2:5anhydro linkage. The hydroxyl group on carbon atom 3 diverges from carbon atom 1 so that a glucosidic ring could not readily be established in that position. Models also showed clearly that no lactone formation can occur in chitonic and chitaric acids and in support of this the two acids retain a constant rotation in aqueous solution.

In the course of examination of the properties of tetramethyl-γ-fructose Haworth, Hirst and Nicholson (J.C.S. 1927, 1513) found that, in contact with dilute hydrochloric acid or by digestion with acetic anhydride in the presence of anhydrous sodium acetate, the compound loses three methoxyl groups. The decomposition product was identified as ω-methoxy-5-methylfurfural and it gave crystalline derivatives with semicarbazide (mp. 166-167° C.) and hydroxylamine (mp. 103-104°C.). By oxidation it was converted to ω-methoxy-5-methylfuran-2-carboxylic acid

(mp. 72-73°C.). This same compound they prepared from chitonic acid by the process indicated as follows:-



Chitonic acid. ω-acetoxy-5-methyl-furan-2-carboxylic acid. ω-methoxy-5-methyl-furan-2-carboxylic acid.

Thus was indicated a close relationship between the structures of chitonic acid and γ-fructose.

In 1933 Zechmeister and Toth (Ber. 66B, 522), in view of the conflicting opinions of Fischer and Tiemann, and Ambrecht concerning the identity of the osazone from chitose, deaminated glucosamine hydrochloride by various methods and in all cases, after addition of phenylhydrazine and acetic acid to the cold solutions, a crystalline by-product rapidly separated in small quantity and was identified as d-arabinosazone which, they considered, was formed from the corresponding free osone present in chitose syrup. On filtering and then heating the clear solution they obtained good yields of glucosazone. They were of the

opinion that the chitosazone reported by Ambrecht was essentially glucosazone containing a small quantity of arabinosazone. They considered that the glucosazone thus prepared could arise from one of three possible sources:-

1. An anhydrohexose.
2. Glucose.
3. Mannose.

The first possibility seemed to them unlikely in view of the fact that, on boiling the deaminated mixture for three hours in a 10% acetic acid solution, the rotatory power remained practically unchanged. They excluded the possibility of the presence of mannose on the grounds that it would have readily combined with phenylhydrazine to give the slightly soluble mannosehydrazone. It was demonstrated that glucosazone separated from the reaction mixture after long standing at room temperature and so they regarded glucose as the chief deamination product. They accounted for the fact that the glucose in chitose syrup was not fermented by yeast by showing that the fermentation of pure glucose is strongly checked by addition of a deaminating mixture.

The existence of chitose as a separate chemical entity was confirmed however by Schorigin and Makarawa-Beneljanskaja (Ber. 68B, 965). They confirmed Zechmeister

and Toth's preparation of glucosazone from chitose. By oxidation of the syrup with nitric acid they obtained only oxalic acid and showed, by control experiments with glucose, mannose and glucosamine, that none of these sugars under similar treatment gave noticeable quantities of oxalic acid. The absence of fructose in the deamination product was indicated by the fact that they obtained no crystalline hydrazone with methylphenylhydrazine. By acetylation they attempted to obtained a crystalline acetyl derivative, but the syrupy product did not crystallise on inoculation with the pentaacetates of glucose and mannose. By treatment of chitose with diphenylhydrazine they isolated in good yield a crystalline hydrazone which differed from the corresponding hydrazones of both glucose and mannose. Control experiments with free glucosamine and glucosamine hydrochloride gave no crystalline derivatives with diphenylhydrazine. Analysis of their chitosediphenylhydrazone indicated that it was probably the hydrazone of an anhydrohexose.

The research work described in this thesis was undertaken with the object of further establishing the identity of chitose and of studying its chemical properties in more detail.

SURVEY OF EXPERIMENTAL RESULTS

and

THEORETICAL DISCUSSION.

d-Arabinosazone and d-glucosazone from chitose solution.

The deamination of glucosamine hydrochloride according to the method of Zechmeister and Toth (Ber. 66B, 552) has been repeated and, in agreement with their report, a small quantity of d-arabinosazone and a considerably larger amount of d-glucosazone has been obtained by treatment of the resulting solution with phenylhydrazine. Furthermore the solution was found to give a strongly positive osone reaction [ARIYAMA's glyoxal reaction (J. Biol. Chem., 74, XLV) found to be positive for solutions containing osones (HYND - unpublished result in this laboratory)], a fact which supports their opinion that some free d-arabinosone exists in the chitose solution. The osone is apparently produced as the result of an oxidation taking place during the process of aeration, by which method they freed the solution from excess nitrous acid, for when carbon dioxide or coal gas was employed in the place of air not only was a very faint osone reaction obtained but no d-arabinosazone could be isolated. It has been demonstrated by HERBST (J. Biol. Chem., 119, 85) that oxidation of glucosamine or glucosaminic acid with chloramine T or other weak oxidising agents

gives rise to the formation of d-arabinose, so that it is not surprising under the conditions of preparation of chitose that a small quantity of d-arabinosone appears in the final solution.

Attempted preparation of crystalline chitose derivatives.

Chitose 2:4dinitrophenylhydrazone.

An attempt at the preparation of chitose 2:4dinitro--phenylhydrazone by adding to a solution of chitose in 50% acetic acid an equivalent quantity of 2:4dinitrophenyl--hydrazine dissolved in glacial acetic acid, leaving the mixed solution overnight at room temperature and then evaporating *in vacuo* at 40°C. to small volume, resulted only in the isolation of the crystalline acetate of 2:4dinitrophenylhydrazine. The corresponding hydrazones of both glucose and mannose were obtained in good yield by this method.

The isolation of crystalline chitose 2:4dinitro--phenylhydrazone was achieved by following the directions of Glasser and Zuckermann (Z. Physiol. Chem., **167**, 44) in their preparation of glucose 2:4dinitrophenylhydrazone. It was obtained in the form of lemon yellow crystalline needles by recrystallising from dry methyl alcohol. The compound melted sharply at 175°C. and a comparison with the melting points of the 2:4dinitrophenylhydrazones of glucose (mp. 119°C.) and mannose (mp. 176°C.) showed that it was identical with neither of these derivatives, the mixed melting point of the chitose and mannose 2:4dinitrophenylhydrazones being 156-160°C.

A dilute solution of the new compound in water gave an intense Molisch's reaction indicating that it contained a carbohydrate residue.

It was demonstrated that the pure substance decomposed fairly rapidly in hot aqueous solution, since, on cooling after boiling for any length of time, a quantity of a red oil separated with the crystalline hydrazone.

Its carbon, hydrogen and nitrogen content determined by microanalysis, as shown in the following table, agreed much more closely with the theoretical values of an anhydrohexose derivative than with those of either normal hexose or pentose derivatives.

	C	H	N
Values determined by microanalysis.	42.22%	4.05%	16.20%
Theoretical for an anhydroxhexose derivative.	42.11%	4.09%	16.37%
Theoretical for a normal hexose derivative.	40.00%	4.44%	15.56%
Theoretical for a normal pentose derivative.	40.00%	4.24%	16.97%

In the course of several preparations it was noted that the yield of chitose 2:4dinitrophenylhydrazone was higher when chitose syrup was dissolved in a cold alcoholic solution of the hydrazine and the resulting solution evaporated <u>in vacuo</u> at 40°C. than in the case where the solution of chitose and 2:4dinitrophenylhydrazine was first boiled for any length of time. From this it was concluded that not only does decomposition occur in hot solution but that the

condensation takes place rapidly at room temperature.

Further evidence was sought for the anhydro structure of chitose in attempts to estimate the number of hydroxyl groups in the chitose 2:4 dinitrophenylhydrazone molecule but methylation of the substance was attended by extensive decomposition and both acetylation and benzoylation resulted in the isolation of amorphous products only.

Chitose condensation products.

No condensation products were obtained when chitose was treated with acetone, benzaldehyde or paraldehyde under the conditions in which the isopropylidine, benzylidine and ethylidene derivatives of glucose are prepared.

Repeated attempts by the Schotten-Baumann reaction have failed to produce the crystalline tribenzoylchitose described by Neuberg, Wolff and Niemann (Ber. 35, 4009).

By acetylation of chitose an amorphous product was prepared which, as shown in the following table, gave analysis figures close to the theoretical values of a pentaacetyl derivative of a normal hexose.

	C	H	$CO.CH_3$
Values found by microanalysis.	49.84%	5.48%	56.4%
Theoretical values for a pentaacetyl hexose.	49.23%	5.64%	55.1%
Theoretical values for a triacetyl anhydrohexose.	50.00%	5.56%	44.8%

Due to the uncertainty regarding the state of purity of the product however, little significance can be attached to such a result although the formation of a pentaacetyl derivative of an anhydrohexose would be in line with the observation of Tiemann and Haarmann (Ber. 19, 1257) that tetraacetyl as well as diacetyl derivatives can be obtained from isosaccharic acid and its diethyl ester.

Methylation of Chitose.

An attempt at simultaneous deacetylation and methylation of the amorphous chitose acetyl derivative with caustic soda solution and dimethyl sulphate resulted in only a very small yield of a dark chloroform soluble product which, on distillation in high vacuum, gave no appreciable quantity of materia distilling at a definite boiling point.

The direct methylation of free chitose by the method Haworth and Leitch (J.C.S., 113, 194) used to prepare tetramethyl β-methylglucoside from free glucose, also resulted in extensive decomposition and the small quantity of dark syrupy product distilled at 0.1 mm. only gradually as the temperature was raised from 120° to 200° C. Part of a small fraction which came over at 145 - 150°C. crystallised, but no other constituent of the mixture was obtained in a condition suitable for examination. The crystalline product, purified by recrystallisation from ethyl acetate melted at 191 - 192°C. It was not obtained in a quantity sufficient for a complete analysis but the facts that analysis indicated the presence of 6.02% nitrogen and that it gave a negative Molisch's reaction indicated that it was probably a methylate glucosamine derivative. This could arise through incomplete deamination of glucosamine in the preparation of chitose solution.

It has not been found possible to repeat the preparation

of the crystalline methyl chitoside (described by Neuberg, Wolff and Niemann, Ber. 35, 4009) by heating chitose in a solution of dry methyl alcohol containing 1½% hydrochloric acid. The colour of the solution darkened so rapidly that the initial fall in rotation could not be followed beyond the preliminary stages, and the syrupy product obtained after neutralisation with silver carbonate and evaporation of the filtered solution at 40°C. in vacuo, showed no sign of crystallisation.

The fact that a solution of chitose does not behave as a free aldehyde towards Schiff's reagent, leads one to suppose that a glucosidic ring structure exists in the molecule. If chitose is 2:5 anhydromannose as seems likely from the work of Levene and La Forge (J. Biol.Chem. 20, 435) one is led to the conclusion from a study of the spatial arrangement of the hydroxyl groups on a molecular model, that a 1:4 glucosidic linkage could exist without undue intramolecular strain. Accordingly one would expect the substance to react as a γ-sugar, though more unstable owing to the additional strain imposed upon the glucosidic ring by the 2:5anhydro ring, and that the glucosidic methyl group could readily be introduced by the action of 1% hydrochloric acid in methyl alcohol at room temperature.

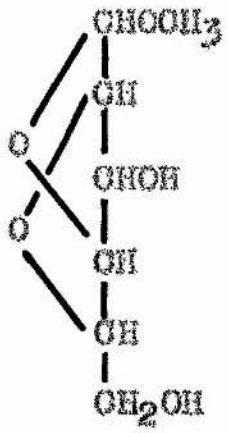
On dissolving 3.75 g. of dry chitose syrup in 75 ml. of dry methyl alcohol containing 1% hydrochloric acid and leaving the solution at room temperature there was observed

a rapid fall in rotation which reached a steady value after 24 hours (Graph 1). By neutralising the cold solution with silver carbonate, filtering and evaporating in vacuo at 40°C. a stiff syrup was obtained which did not crystallise and which reduced Fehling's solution to only a comparatively slight extent. It was assumed that this syrup was a mixture of Neuberg's methyl chitoside, some free chitose and a quantity of decomposition products, and that on further methylation and subsequent fractional distillation of the product in high vacuum, a comparatively pure fully methylated chitose derivative could be obtained. The methylation by the method of Haworth proceeded without extensive decomposition and the main product was obtained as an almost colourless mobile liquid boiling at 100°C./0.1 mm. It gave analysis figures and molecular weight indicating the formula $C_{10}H_{19}O_5$ and had a methoxyl content of 56.7%. A sample of methyl-chitoside syrup was also methylated by the method of Purdie and the same product distilling at 100°C./0.1 mm. and having a methoxyl content of 56.8% was obtained.

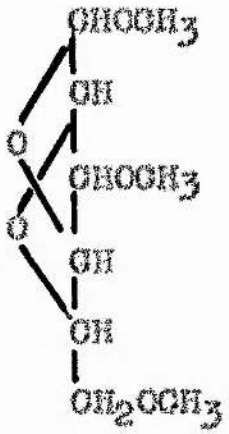
The methylated product was quite soluble in water, the solution having a rotation of + 28.20°. Its neutral aqueous solution did not reduce Fehling's solution but after heating with a little dilute hydrochloric acid it reduced Fehling's solution strongly.

If methyl chitoside has the structure A one would expect that complete methylation would result in the trimethe

derivative B which has a methoxyl content of 45.6%.

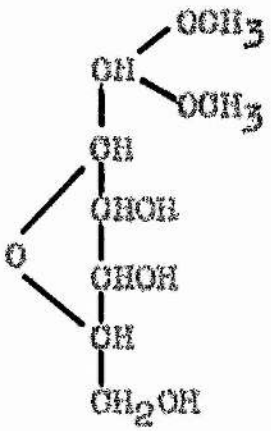


A.
Methyl chitoside.

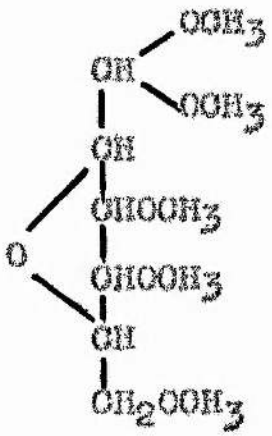


B.
Dimethyl methyl chitoside.

Another possibility may arise from the fact that, owing to the apparent strain to which the 1:4 glucosidic structure is subjected by the 2:5anhydro ring, the treatment of chitose with methyl alcoholic hydrochloric acid may give rise to chitose dimethylacetal C as the main reaction product which on methylation should be converted to the pentamethoxy derivative D. This has a methoxyl content of 62.0%.

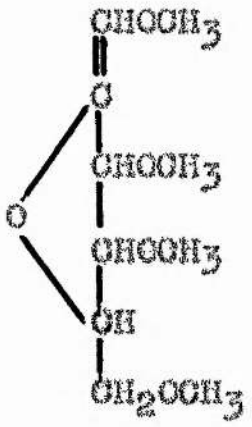


C.
Chitose dimethyl acetal.



D.
Trimethylchitose dimethylaceta

The actual methoxyl content of the product lies midway between the theoretical values of the two substances B and D so that there remains the possibility of its being a tetramethoxy derivative. The only structure which can be devised for a fully methylated tetramethoxy 2:5anhydro-hexose is that of the unsaturated compound E.



E.

This could conceivably arise by cleavage of the glucosidic ring and a simultaneous splitting off of water to produce the 1:2 unsaturated linkage during the methylation of methyl chitoside. It has a theoretical carbon, hydrogen and methoxyl content in reasonably close agreement with the values estimated. Further grounds for the consideration of such a possibility were demonstrated by the fact that a strong aqueous solution of the syrup immediately decolourise bromine water and permanganate solution and that the molecul weight determination gave a figure (218.1) close to the

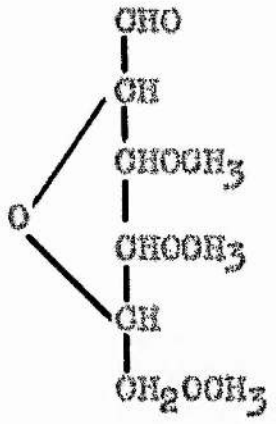
theoretical value of E (218).

The difficulty encountered in the case of a material such as this methylated product, is that no sharp criterion of purity can be obtained and in spite of the fact that it distils at a fixed temperature in high vacuum it may still contain a degree of impurity especially as it is derived from the apparently complex mixture of chitose and its decomposition products. A search was therefore made for a crystalline derivative of the methylated product which could be purified and subjected to accurate analysis.

Structure of fully methylated chitose.

Hydrolysis of fully methylated chitose was attempted in aqueous N/100 hydrochloric acid solution but the course of the reaction could not be followed polarimetrically beyond the initial fall in rotation owing to the appearance of a fine precipitate. The hydrolysis was therefore carried out as for methylated γ-methyl glucosides by heating a 5% solution in N/10 hydrochloric acid for 2 hours at 100°C. The neutralised solution readily reduced Fehling's solution and rapidly restored the colour to Schiff's reagent indicating the presence of a free aldehyde. Evaporation of the solution at 40°C. in vacuo resulted in a moderately mobile syrup which failed to crystallise. The material was purified to some extent by distillation, the main fraction coming over at 120°C/0.1 mm., and this product gave analysis figures in close agreement with the theoretical values of trimethyl aldehydochitose F.

	C.	H.	.OCH3
Values obtained by analysis.	52.18%	7.85%.	45.4%.
Theoretical for trimethyl aldehydochitose.	52.94%	7.84%	45.6%



E.

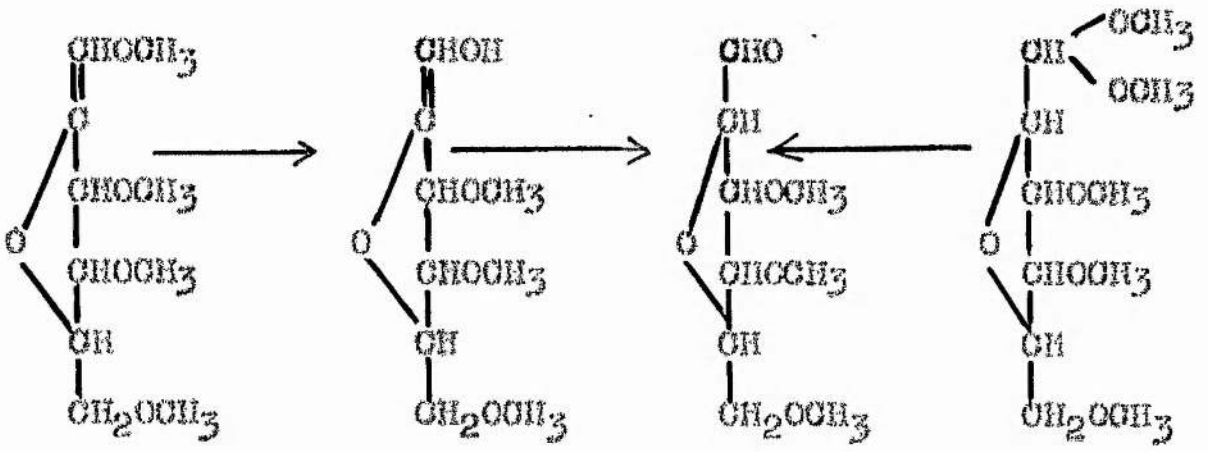
Trimethyl aldehydochitose.

On dissolving trimethyl aldehydochitose syrup in an aqueous solution containing phenylhydrazine and acetic acid a light red viscous oil rapidly separated at room temperature but the product failed to crystallise and invariably separated as an oil from a large number of solvents. Attempts to prepare the 2:4dinitrophenylhydrazine derivative in crystalline form were also unsuccessful.

The semicarbazone however readily crystallised in the form of long white needles and was purified by recrystallisation from methyl alcohol. It melted sharply at 148°C. and the following analysis figures indicated that it was the semicarbazone of trimethyl aldehydochitose.

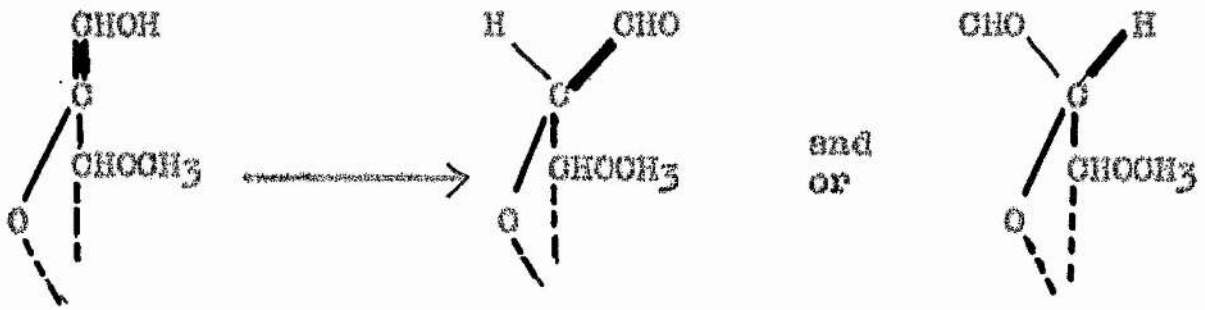
	C	H	N	.OCH3
Results obtained by analysis.	46.15%	7.32%	15.90%	35.4%
Theoretical for trimethyl aldehydochitose semicarbazone.	45.98%	7.28%	16.09%	35.6%

Trimethyl aldehydochitose could be derivated by hydrolysis from either of the two possible structures E and D postulated for fully methylated chitose as indicated by the following structural changes:-



Substance E. Trimethyl aldehydochitose. Substance D.

The first alternative, involving the hydrolysis of E to trimethyl aldehydochitose through the hypothetical intermediate enol form, would appear more probable from the analysis and preliminary examination of fully methylated chitose and if this were the case, de-enolisation of the intermediate hydrolysis product should give either one or a mixture of two possible isomers as is obvious from an examination of the following structures:-



One of these cis-trans isomers is trimethyl aldehydochitose. The other is trimethyl aldehydoepichitose. Only one semi-carbazone, however was isolated from the hydrolysis product.

It was hoped that oxidation of fully methylated chitose with permanganate would yield products which might provide confirmation of the unsaturated structure provisionally ascribed to this compound. One would expect that it would almost immediately decolourise a solution of potassium permanganate equivalent to one atomic proportion of oxygen, but it was found on titration with neutral, acid or alkaline permanganate, that in each case only the first few drops of the solution were immediately decolourised after which the rate of oxidation proceeded very slowly. Moreover it has been demonstrated, by plotting on a graph (Graph 2) the time of reaction at constant temperature against the permanganate content of a solution containing the compound and a quantity of potassium permanganate equivalent to 20 atomic proportions of oxygen, that at no stage was there any sudden decrease in the rate of oxidation at which one might expect to encounter a single and comparatively stable intermediate oxidation product.

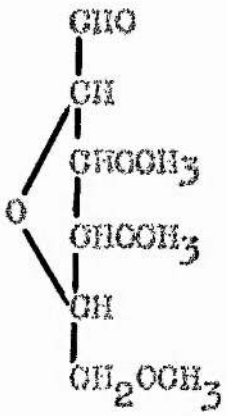
The fact that permanganate solution equivalent to one atomic proportion of oxygen is not rapidly decolourised by fully methylated chitose, throws doubt on the hypothesis that an ethylenic linkage is incorporated in the molecule. A number of attempts were therefore made at quantitative estimation of the unsaturated linkage using both Wij's metho and the bromine addition method but in every case only a negligible unsaturation value was obtained.

If, as seems certain from these results, fully methylat chitose has no double bond in its molecule, it cannot be a tetramethoxy derivative since the unsaturated structure E is the only one which could possibly be ascribed to a tetramethoxy 2:5anhydrohexose which was completely methylate One is therefore forced to the conclusion that the material is not a single chemical entity but a mixture containing trimethylchitose dimethylacetal as one of the main constituents.

Attempted preparation of trimethyl aldehydochitose by deamination of 3:4:6trimethyl glucosamine hydrochloride.

To trimethyl aldehydochitose, from which was prepared the above-mentioned crystalline semicarbazone, has been provisionally ascribed the structure of a 3:4:6trimethyl

2:5anhydro-aldehydohexose:-

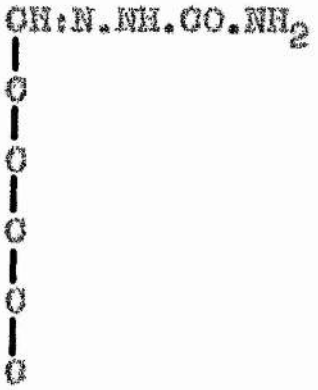


One would expect that this compound would be produced by deamination of 3:4:6trimethyl glucosamine hydrochloride just as chitose results from the deamination of glucosamine hydrochloride. If this were the case, it would provide strong evidence in support of the above structure. However, when 3:4:6trimethyl glucosamine hydrochloride (prepared by the method of White, J.C.S., 1940, 443) was deaminated either by silver nitrite or sodium nitrite, the product, on treatment with semicarbazide, yielded no trimethyl aldehydochitose semicarbazone but a different crystalline semicarbazone melting at 166°C. It was considered most likely that in the deamination of glucosamine hydrochloride and 3:4:6trimethyl glucosamine hydrochloride whereas in one case it was accompanied by a Walden inversion on carbon atom 2, in the other no inversion was involved so that the new compound would be the stereoisomeric semicarbazone of trimethyl aldehydoepichitose. The following results of

analysis however did not bear out this hypothesis.

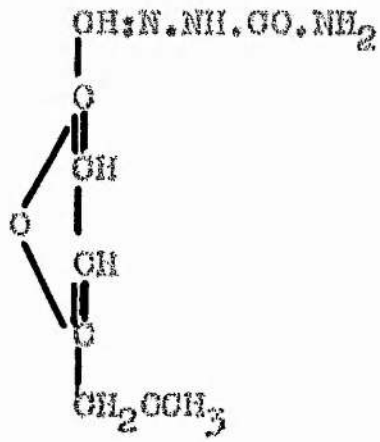
	C	H	N	OCH_3
Values found by analysis.	48.91%	5.74%	20.90%	14.6%
Theoretical for trimethyl aldehydoepichitose semi-carbazone.	45.98%	7.28%	16.09%	35.6%

The analysis figures indicated the formula of the substance to be $C_8H_{11}N_3O_3$. The compound being a semicarbazone must incorporate the grouping .CH:N.NH.CO.NH_2 and furthermore, if the six carbon chain of glucosamine has not been severed in the deamination, it will probably have the skeletal structure:-



The methoxyl content of 14.6% would be given by a substance of molecular weight 198 containing only one methoxyl group so that apparently two methoxyl groups have been lost in the deamination process. If this demethoxylation had occurred on carbon atoms 3 and 4 one could write:-

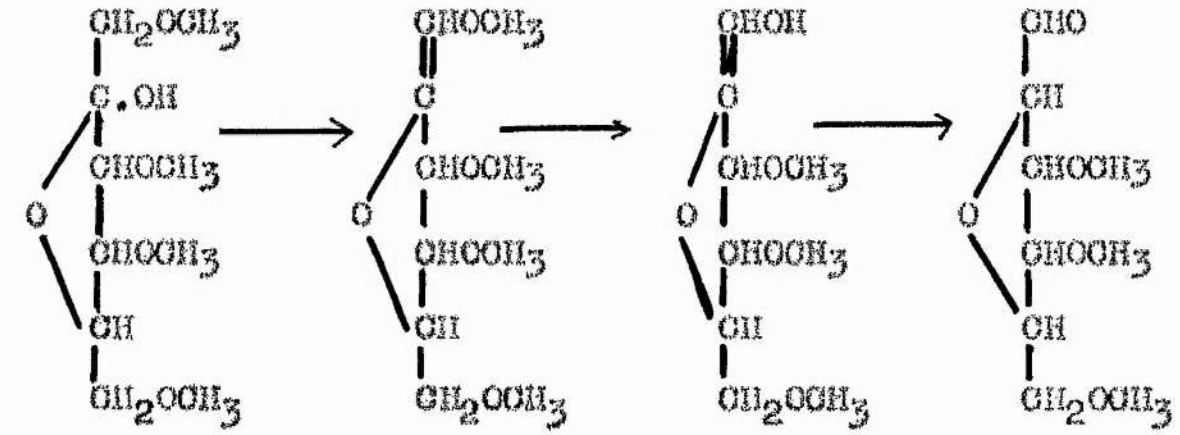
leaving H_2O to be incorporated into the molecule. The only possibility on these assumptions appears to be a substance having the following structure:-



This compound, the semicarbazone of ω-methoxy-5-methyl-furfural, has been previously prepared, notably by Haworth, Hirst and Nicholson (J.C.S., 1927, 1513) who isolated it from the reaction product obtained by digesting tetramethyl γ-fructose with 8% hydrochloric acid. For the purpose of comparison a specimen of the compound was prepared from tetramethyl γ-fructose under the conditions described by those workers. It melted at 166°C. and no depression of melting point was noted on mixing it with the semicarbazone

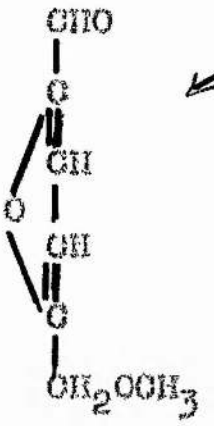
of the 3:4:6trimethyl glucosamine hydrochloride deamination product.

It was considered likely by Haworth, Hirst and Nicholson that the reaction in which tetramethyl γ-fructose was converted to ω-methyoxy-5-methyl-furfural followed the course indicated thus:-



Tetramethyl-γ-fructose.

Trimethyl aldehydochitose.

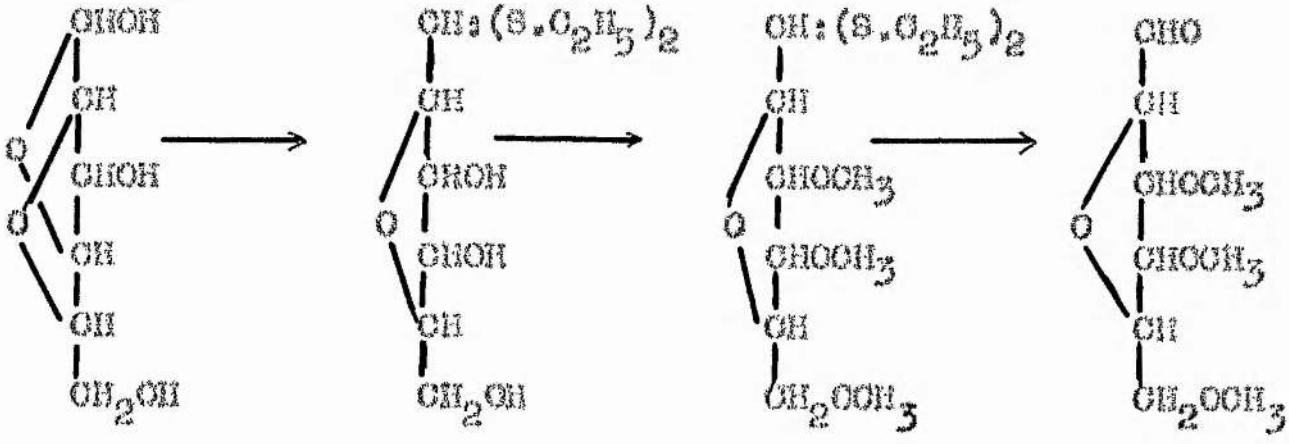


ω-methoxy-5-methyl-furfural.

This involves the formation of trimethyl aldehydochitose as an intermediate product but the latter, prepared from chitose as previously described, gave no ω-methoxy-5-methyl-furfural when it was digested with 8% hydrochloric acid.

Preparation of trimethyl aldehydochitose from chitose diethyl mercaptal.

Strong evidence that the hydrolysis of fully methylated chitose results in the formation of trimethyl aldehydochitose has been obtained in the preparation of the latter from chitose by the following series of reactions which represent the usual method employed in the conversion of aldohexoses to the corresponding methylated aldehydohexoses (Levene and Meyer, J. Biol. Chem., 69, 175).



Chitose. | Chitose diethyl mercaptal. | Trimethylchitose diethylmercaptal. | Trimethyl aldehydo-chitose.

Chitose was found to combine readily with diethylmercaptan by the usual method employed in the preparation of sugar mercaptal derivatives (Fischer, Ber. 27, 674) and the condensation product was extracted with ethyl acetate. Removal of the solvent at 35°C. in vacuo resulted in a good yield of a viscous syrup which did not crystallise but which gave the following analysis figures in close agreement with the

theoretical values.

	C	H	S
Values found by analysis.	44.83%	7.43%	22.95%
Theoretical for chitose-diethylmercaptal.	44.78%	7.46%	23.88%

An attempt at methylation of the syrup by Purdie's method resulted in extensive decomposition. On methylation of chitose diethylmercaptal by the method of Haworth and distillation of the product in high vacuum, a light yellow distillate, boiling at 150-160°C./0.12 mm., was obtained in good yield. This was subjected to a second Haworth methylation and again the product distilled, the bulk of the material boiling at 145-153°C./0.12 mm. Estimation of the methoxyl content of this material gave the figure 24.3% instead of 30.0% as calculated for trimethylchitose diethylmercaptal. Incomplete methylation was also indicated by the gradual rise in distillation temperature of the product. The syrup was subjected to three further methylations by the more drastic method of Freudenberg and as a result a light yellow mobile liquid was obtained which distilled at 135°C./0.12 mm. exhibited a rotation in acetone solution of + 32.16°, and gave the following analysis:-

	C	H	S	.OCH$_3$
Values found by analysis.	51.20%	8.39%	20.71%	29.7%
Theoretical for trimethyl chitose diethylmercaptal.	50.32%	8.39%	20.65%	30.0%

Removal of the mercaptan groups from trimethylchitose diethylmercaptal with mercuric chloride resulted in the formation of a mobile syrup which, by addition of semicarbazide gave the crystalline trimethyl aldehydochitose semicarbazone previously described.

Oxidation of fully methylated chitose with nitric acid.

It was hoped that further evidence in support of the hypothesis that fully methylated chitose is a trimethyl 2:5anhydrohexose derivative would be obtained by its oxidation with nitric acid and isolation of substances of known constitution. The main oxidation product was obtained as the methyl ester of an organic acid by esterification and distillation in high vacuum. It distilled at 100°C./0.1 mm. as a colourless mobile syrup which did not crystallise and was found to have a rotation in methyl alcohol of + 52.09°. Analysis of the material gave the following data:-

C 50.00%

H 7.45%

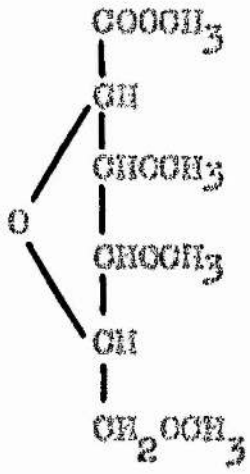
.OCH$_3$ 51.1%

Equiv. wt. ... 220.

The molecular weight of fully methylated chitose was estimated as 218 so that the molecular weight of the methyl ester of its oxidation product could not be much greater than this value. The formula calculated from its carbon and hydrogen content is $(C_5H_9O_3)_n$ so that the only possibilities on these grounds are $C_5H_9O_3$ and $C_{10}H_{18}O_6$, the molecular weight of the former being 117 and of the latter 234. The formula $C_5H_9O_3$ is ruled out by the estimated equivalent weight (220) being almost twice the molecular weight of such a compound so that the only possibility is $C_{10}H_{18}O_6$, the methyl ester of a monocarboxylic acid. From the supposition that the six membered carbon chain of chitose has remained intact, one can ascribe to the compound the skeletal structure:-

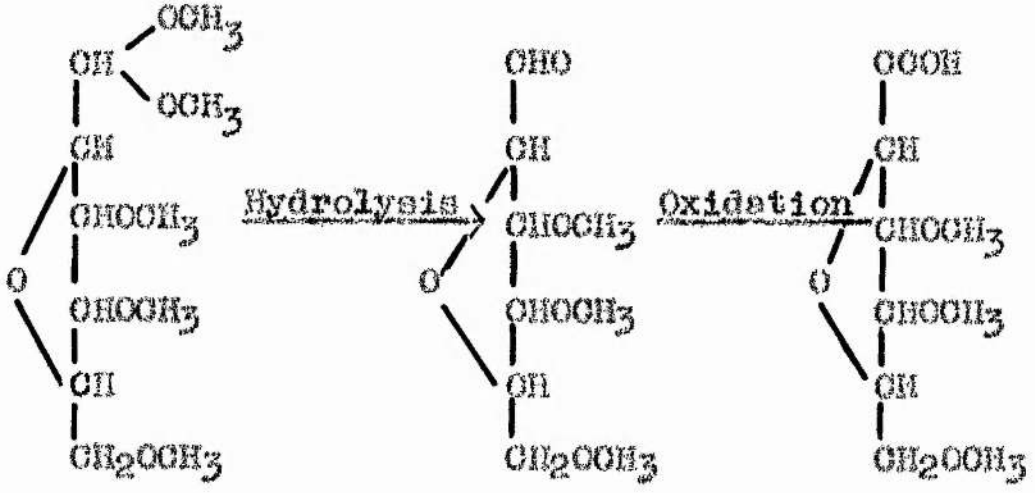
leaving three of the carbon atoms to be accounted for.

These are probably present as methoxyl groups, leading one to the conclusion that the substance is the methyl ester of trimethyl chitonic acid having the formula $C_{10}H_{18}O_6$ and the probable structure:-



The theoretical methoxyl content of this compound is 53.0% close to the value estimated for the syrup.

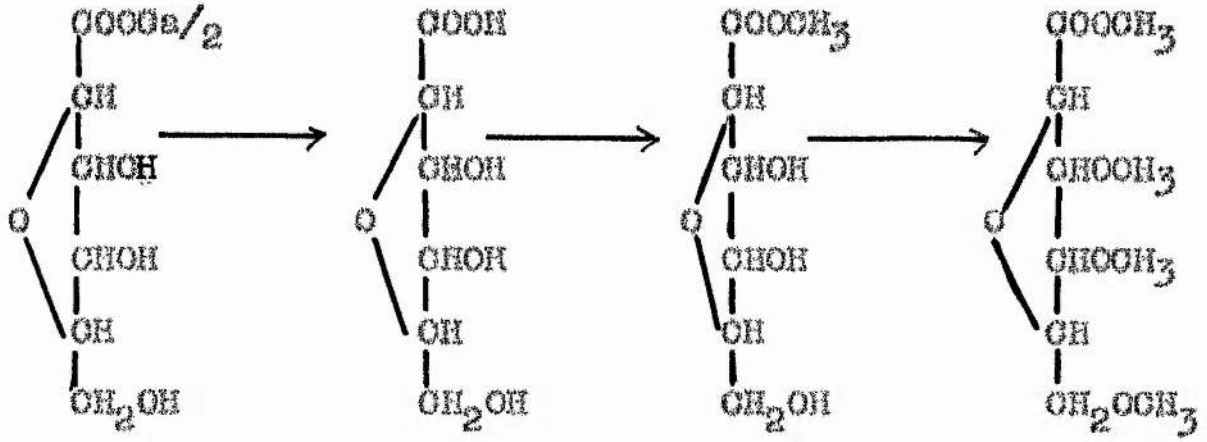
That trimethyl chitonic acid could be derived by simple hydrolysis and oxidation of trimethylchitose dimethylacetal is obvious from the following structural changes:-



From the methyl ester the free acid, its amide and calcium salt were prepared but none of these was obtained in crystalline form suitable for more exact analysis.

Preparation of trimethyl chitonic acid by methylation of Chitonic acid.

For the purpose of comparison the preparation of the methyl ester of trimethyl chitonic acid from pure crystallin calcium chitonate was undertaken by the series of reactions indicated thus:-



Calcium chitonate. | Chitonic acid. | Methyl chitonate. | Trimethyl meth chitonate.

Chitonic acid prepared as the uncrystallisable syrup described by Fischer and Tiemann (Ber. 27, 128) was esterified in methylalcoholic hydrochloric acid solution. The ester was isolated by evaporation in vacuo to a viscous syrup and

extraction from unchanged chitonic acid with ethyl acetate. The substance obtained by evaporation of this extract was a colourless syrup which did not crystallise but it gave the following analysis figures close to the theoretical values for methyl chitonate.

	C	H	.OCH3
Values found by analysis.	43.65%	6.28%	15.9%
Theoretical for methyl chitonate.	43.75%	6.25%	16.15%

The ethyl ester prepared in a similar manner crystallised readily and was purified by recrystallisation from iso-propyl alcohol. It had a melting point of 106°C. The rotation of its solution in absolute alcohol was + 46.69°. Analysis:-

	C	H	Equivalent wt.
Values found by analysis.	46.58%	6.90%	204
Theoretical for ethyl chitonate.	46.60%	6.80%	206

The methylation of methyl chitonic ester by the Purdie method proceeded normally and resulted in a colourless mobile syrup distilling at 100-103°C./0.1 mm. and having a rotation in methyl alcohol of + 55.35°. Analysis:-

	C	H	.OCH3	Equivalent wt.
Values found by analysis.	52.00%	7.68%	52.2%	230.
Theoretical for methyl trimethylchitonate.	51.28%	7.69%	53.0%	234.

A comparison of the substance with the methyl ester of the acid obtained by oxidation of fully methylated chitose is shown in the table below.

	Trimethyl methyl chitonate.	Methyl ester of the oxidation product.
Appearance.	Mobile liquid	Mobile liquid.
Boiling point.	100-103°C./0.1 mm.	100-103°C./0.1 mm.
Rotation (aq.soln)	+ 55.35°	+ 52.09°
Equivalent wt.	230	220
Calcium salt	Amorphous	Amorphous
Amide	Viscous syrup	Viscous syrup
Free acid	Viscous syrup	Viscous syrup.

Although none of the derivatives were isolated in pure crystalline form, this comparison of their properties confirmed the probability that the two esters were identical. The observed differences could reasonably be accounted for by the presence, in the ester of the oxidation product, of impurities which might well be expected from its drastic method of preparation. The sodium salt and the phenylhydrazide of trimethyl chitonic acid were also prepared but again only amorphous products resulted.

Examination of 'methyl chitoside' syrup.

The experimental evidence thus far obtained lends support to the hypothesis that fully methylated chitose syrup contains trimethylchitose dimethylacetal as one of the main constituents. The low methoxyl content of the substance could be accounted for by the presence either of the fully methylated dimethyl α- and β-methyl chitosides or of methylated chitose decomposition products. In the hope of clearing up this uncertainty a study of 'methyl chitoside' syrup was undertaken. From an examination of its solubility in a number of solvents it was found possible to separate 'methyl chitoside' syrup into three distinct fractions thus:-

F_1 : Insoluble in ethyl acetate (approx. $\frac{1}{3}$ the total wt.).

F_2 : Soluble in ethyl acetate - insoluble in ether. (approx. $\frac{1}{3}$ the total wt.).

F_3 : Soluble in ethyl acetate - soluble in ether. (approx. $\frac{1}{6}$ the total wt.).

Hydrolysis of the three syrups thus isolated and subsequent treatment of each with 2:4dinitrophenylhydrazine resulted in the preparation of crystalline chitose 2:4dinitrophenylhydrazone from F_2 only, dark uncrystallisable oily products being obtained from both F_1 and F_3. It seemed probable from this result that the ethyl acetate soluble - ether insoluble

fraction was the only one containing any considerable quantity of chitose methyl alcohol condensation products, and that F_1 and F_3 were made up for the most part of chitose decomposition products.

F_2 was found to have a methoxyl content of 24.3% intermediate between the theoretical values of methyl chitoside (17.6%) and chitose dimethylacetal (29.8%), so that the material might be either a mixture of these two substances or a mixture of chitose dimethylacetal and chitose decomposition products.

One would expect that a mixture of chitose dimethylacetal and methyl chitoside would be converted almost completely to the former by further treatment with dry methylalcoholic hydrochloric acid solution, but the methoxyl content of the syrup (24.8%) obtained from F_2 by such treatment had not increased to any significant extent. It seemed likely, therefore, that F_2 was essentially a mixture of chitose dimethylacetal and chitose decomposition products containing little or no methyl chitoside.

These results support the view that chitose solution as obtained by the deamination of glucosamine hydrochloride is a complex mixture of chitose and other products. It is apparent that in the study of its condensation with methyl alcohol and other properties it would be of advantage to begin with a much purer sample of chitose than has been hitherto obtained. With this object in view it was therefor

decided first to attempt to devise a method for estimating chitose with reasonable accuracy, then to make a study of its stability under varying conditions and finally to estabi conditions under which glucosamine hydrochloride could be deaminated almost completely to yield chitose free as far as possible from decomposition or reaction by-products.

Estimation of chitose.

The method of Hanes (Biochem. J., 23, 99) as used in the determination of reducing sugars, was found to be inapplicable in this case for the extent of ferricyanide reduction was not proportional to the volume of chitose solution added (Graph 3).

From the result of examination of the rate of oxidation of chitose by Hanes' solution at 70°C. (Graph 4) it was evident that after the initial rapid oxidation, probably representing for the most part the transformation of chitose to chitonic acid, there continued a slower secondary oxidation which proceeded at a rate approximately proportional to the amount of chitose solution added. This secondary oxidation might represent a further degradation of chitonic acid or an oxidation of chitose decomposition products apparently present in considerable quantity in chitose solution.

An attempted modification of Hanes' method, by buffering the solution with sodium acetate in place of sodium carbonate proved unsuccessful. It was hoped that by thus lowering the pH of the reaction medium the secondary oxidations might be eliminated but, as is seen from Graph 5, the chitose oxidation rate was unduly retarded.

It was considered that the yields of chitose 2:4dinitro-

phenylhydrazone obtained from chitose solutions in previous experiments gave in each case a rough indication of the chitose content, and attempts were made to adapt this reaction to estimate the chitose contained in a small volume of solution. When the condensation was carried out by dissolving chitose in an alcoholic solution of 2:4 dinitrophenylhydrazine and evaporating the solution to small bulk, the product was obtained as a red oil from which the hydrazo gradually crystallised. Difficulty is experienced in separating the crystals from such an uncrystallisable oily residue as remained in this case without loss of material. However it was found that the hydrazone separated in the form of clean yellow crystals when an aqueous chitose solution was shaken for some time with a saturated solution of the hydrazine in cold nitrobenzene. Since, at room temperature, 2:4dinitrophenylhydrazine is fairly soluble in nitrobenzene and almost completely insoluble in water while chitose 2:4dinitrophenylhydrazone is very sparingly soluble in both these solvents, filtration of the product after completion of the condensation should give an almost quantitative yield of the chitose hydrazone. The value of the chitose content of a solution thus estimated agreed remarkably well with that obtained by a second method carried out by long shaking of the cold aqueous solution with a weighed excess of finely powdered 2:4dinitrophenylhydrazine and by calculating the chitose content from the

increase in weight of the filtered and dried product, essentially a mixture of crystalline chitose 2:4 dinitrophenylhydrazone and unchanged 2:4dinitrophenylhydrazine. This second method obviated the necessity for using relative large volumes of nitrobenzene to keep the excess of unchange hydrazine in solution. It was checked by washing out the excess 2:4dinitrophenylhydrazine with nitrobenzene, leaving a reasonably pure sample of chitose 2:4dinitrophenylhydrazon the dry weight of which corresponded closely with the value estimated from the weight of the mixed product.

The rate of condensation in this method was determined by shaking each of a number of small measured volumes (0.5 n of a chitose solution with an excess of the powdered reagent for different lengths of time, and measuring the increase in weight of the dried solid product in each case (Graph 6).

Reasonably close duplicate values (within 2%) were obtained from those mixtures which had been shaken for a sufficient length of time. The main disadvantage arose from the fact that, owing to the insolubility of 2:4dinitrophenylhydrazine in water, the mixture required shaking for at least 40 hours before the reaction was completed.

In the hope of obtaining a similar but more rapid method of estimation a search for further crystalline hydrazones of chitose was conducted. The condensation of chitose in cold aqueous solution with phenylhydrazine, p-tolylhydrazine, p-bromophenylhydrazine, methylphenyl-

hydrazine and phenylhydrazine-p-sulphonic acid resulted in the isolation of uncrystallisable oily products only.

Chitose benzylphenylhydrazone separated as a fine white crystalline mass when an aqueous solution of chitose was shaken at room temperature with benzylphenylhydrazine dissolved in benzene. The compound, purified by recrystalli ing from aqueous alcohol, melted sharply at 85°C., and had a constant rotation in absolut alocohol of + 50.25°. Microanalysis of this compound has apparently presented some difficulty for in four estimations the following range of results were obtained.

	C.	H.	N
	63.18% -64.15%	6.34% -7.33%	7.24-8.7
Calculated for an anhydro-hexose derivative.	66.67%	6.43%	8.19%

A crystalline chitose hydrazone was also prepared with p-nitrophenylhydrazine and was purified by recrystallising from absolute alcohol. The compound melted sharply at 185°C. It had a constant rotation in absolute alcohol of + 7.09°.

Analysis:-

	C	H	N
Values found by micro-analysis.	48.35%.	5.00%.	14.15
Theoretical for an anhydro-hexose derivative.	48.48%	5.05%	14.14
Theoretical for a normal hexose derivative.	45.71%	5.40%	13.33

Attempts at estimation of the chitose content of a solution by shaking it with an excess of benzylphenylhydrazi dissolved in benzene, filtering, drying and weighing the crystalline product, indicated that the reaction was much more rapid (complete after 5 hours shaking) than when 2:4dinitrophenylhydrazine was employed. Difficulty was experienced however in filtering off and cleaning the finely matted crystalline product and a difference in value as grea as 10% was obtained for duplicate estimations.

By using pure finely ground p-nitrophenylhydrazine in place of 2:4dinitrophenylhydrazine the condensation proceede comparatively rapidly, the separation of the product being complete after 2-3 hours (Graph 7). Close duplicate values (within 2%) were obtained using as little as 1 ml. of chitose solution (containing 0.1 g. of deaminated glucosamine hydrochloride, i.e., a theoretical chitose content of 0.075 g.) though the values were 2-3% lower than those obtai by using 2:4dinitrophenylhydrazine due to the greater solubil ity of p-nitrophenylhydrazine in water. However, by

determining the solubilities of p-nitrophenylhydrazine and its chitose hydrazone in water at room temperature, and making a correction for these factors in measuring the increase in weight of the solid, the value estimated for the chitose content of a solution was in close agreement wit that obtained by the 2:4dinitrophenylhydrazine method.

In view of the possibility of the presence of some glucose or mannose and undeaminated glucosamine hydrochlorid in chitose solutions, the effect of shaking aqueous solution of these sugars with p-nitrophenylhydrazine for 3 hours at room temperature was investigated. Since in none of these cases did condensation occur to any appreciable extent, the presence of comparatively small amounts of these sugars woul not interfere with this method of chitose estimation.

The amount of d-arabinosone in chitose solution, taken as that calculated from the highest yield of d-arabinosazone obtained by the action of phenylhydrazine and acetic acid on the cold solution, was not sufficient to appreciably effect the estimation of chitose by this new method since the increase in weight of the solid due to arabinose p-nitrophenylosazone formation would be within the experimental error involved in the estimation.

Examination of the stability of chitose.

A reasonably accurate method for the estimation of chitose in aqueous solution having been devised, attention was directed to a study of the stability of chitose under varying conditions.

(I) It was found that vigorous aeration of a solution at room temperature for two hours did not affect its chitose content to any appreciable extent.

(II) After heating a solution of chitose for half an hour at 90°C. the extent of chitose decomposition was estimated to be about 20%.

(III) Considerable decomposition, amounting to 35%, occurred when the chitose solution was evaporated *in vacuo* at 40°C. to a stiff syrup, but when the evaporation was interrupted at a point where the product was obtained as a moderately mobile syrup, the extent of decomposition was only 8 - 9%.

(IV) After evaporating the chitose solution in a vacuum desiccator over concentrated sulphuric acid at 0°C. to a stiff syrup, it was estimated that 16% of the chitose had undergone decomposition but the extent of decomposition under these conditions was markedly affected by a variation of the pH value of the solution as indicated below.

(V) The amount of chitose in 10 ml. of the solution made practically neutral to litmus by addition of sodium

acetate and evaporated in vacuo at 0°C. to a viscous syrup was decreased by 25%.

(VI) The extent of decomposition on evaporation of solutions varying in acidity from one made faintly acid with a drop of glacial acetic acid to one containing 10% acetic acid was found to be 16 - 18%.

(VII) Almost complete decomposition occurred when a chitose solution containing 1% hydrochloric acid was evaporated under the same conditions.

Deamination of glucosamine hydrochloride.

In this series of experiments the extent of deamination was calculated from the volume of nitrogen evolved from a known weight of glucosamine hydrochloride.

(1) Deamination with sodium nitrite.

A solution of glucosamine hydrochloride on treatment with an equivalent weight of sodium nitrite evolved nitrogen equivalent to 84.4% deamination.

By acidifying a similar solution with a drop of acetic acid the rate of deamination was markedly increased, but the final volume of liberated nitrogen indicated that only 75.9% of the glucosamine had been deaminated. From the fact that nitrogen evolution recommenced when a small quantity of sodium nitrite was added to this solution, it was considered that the incomplete deamination in these cases was the result of a loss of a quantity of the volatile nitrous acid, particularly in acid solution, during the process of nitrogen evolution.

Deamination with 1½ molecular proportions of sodium nitrite in a solution at -2° to -3°C. acidified with a quantity of acetic acid equivalent to the excess of nitrite used, resulted in the evolution of almost 99% of the theoretical volume of nitrogen and the production of 93.7% of the theoretical yield of chitose.

(2) Deamination with silver nitrite.

By employing silver nitrite in place of sodium nitrite only 77% deamination was obtained in an experiment conducted according to the directions of Zechmeister and Toth (Ber., 66B, 552). A more complete reaction resulted though still less than 90% of the theoretical volume of nitrogen was evolved when the deamination mixtures were acidified with acetic acid.

An almost complete deamination (97.3%) was obtained with silver nitrite when the mixture was acidified with hydrochloric instead of acetic acid, but the yield of chitose thus obtained was only 7l% of the theoretical quantity.

Preparation of chitose from chitose 2:4dinitrophenylhydrazine.

A solution of chitose was prepared by the action of benzaldehyde on a solution of pure chitose 2:4dinitrophenylhydrazone, the yield obtained being 73.8% of the theoretical quantity. This product on treatment with phenylhydrazine in acetic acid solution was converted to d-glucosazone thus establishing beyond doubt the fact that d-glucosazone, prepared from a solution of chitose formed by the deamination of glucosamine hydrochloride, is not solely derived from the undeaminated glucosamine present.

Preparation of dry amorphous chitose.

20 ml. of the solution of chitose prepared by deaminati of glucosamine hydrochloride solution with 1½ molecular proportions of sodium nitrite and ½ molecular proportion of acetic acid at -2 to -3°C. and estimated to contain 1.404 g. (93.4% of the theoretical quantity) of chitose, was evaporated under diminished pressure at 23-25°C. to a moderately mobile syrup. The product was dehydrated by repeated extraction with pure acetone and the amorphous residue of practically dry chitose was freed from traces of acetone by placing it under high vacuum at 20°C. for 10 minu To what extent the chitose had undergone decomposition durin these processes was determined by dissolving in water, makin up to 20 ml. in a graduated flask and carrying out the chito estimation method on the solution. 91.7% of the original quantity of chitose was found to be present or 85.6% of the theoretical amount obtainable from the glucosamine hydrochloride deaminated.

Condensation of chitose with methyl alcohol.

Having obtained a suitable method for the preparation of dry chitose in good yield and containing practically no undeaminated glucosamine, further investigations were made into its condensation with methyl alcohol.

Methyl chitoside.

The rotation of an almost neutral solution of approximately 5% chitose in dry methyl alcohol at room temperature was observed over a period of 5 days but apart from a very small initial increase the rotatory power remained unaltered. At the same time the reducing power of the solution was tested by adding 0.5 ml. to 2 ml. of cold Fehling's solution and observing the deposit of cuprous oxide which, after a few hours, had completely settled at the bottom of the test tube. A considerable deposit was obtained in a test on the freshly prepared solution but the amount was markedly reduced after the solution had been left at room temperature for 24 hours. No further decrease in reducing power was noted after the solution had been left at room temperature for a further period of 4 days. From this observed decrease in reducing power of the solution it was considered that condensation of chitose and methyl alcohol had occurred with the formation of either methyl chitoside or chitose dimethylacetal. A test portion of the solution, made faintly alkaline by the addition of a very small quantity of anhydrous sodium carbonate which,

it was hoped, would reduce hydrolysis to a minimum, was evaporated *in vacuo* at low temperature to a white brittle amorphous solid of constant weight. The methoxyl content of this material (6.8%) confirmed the suspicion that a certa degree of condensation had taken place. Attempts at separat ion of the methylated product from unchanged chitose by extraction with various solvents were unsuccessful. The fact that the material contained no ethyl acetate soluble portion showed that no chitose dimethylacetal was present.

It was realised that the presence of small amounts of sodium acetate and sodium carbonate in the product isolated in the above experiment might be responsible for a considerable proportion of the estimated methoxyl content by virtue of the fact that they probably retain a quantity of methyl alcohol of crystallisation. The effect of the presence of these salts was examined by adding an excess of anhydrous sodium carbonate to a 1% solution of acetic acid in dry methyl alcohol and evaporating the resulting alkaline solution to a dry solid mass under the conditions described for the isolation of the chitose condensation product. Estimation of the methoxyl content of the salt mixture (4.6% and calculation of the quantities present in the methyl chitoside preparation showed that only a small fraction of the methyl iodide liberated in the methoxyl estimation of 'methyl chitoside' could be derived from this source.

The addition of a small amount of glacial acetic acid to the remainder of the chitose in methyl alcohol giving a

solution distinctly acid to litmus and containing approximate 0.025% acetic acid did not affect the rotatory power over a period of three days and there was no marked decrease in the reducing power of the solution. The product however isolated from a test portion of the solution as in the previous case was found to have increased in methoxyl content to 10.2%. Further additions of glacial acetic acid (up to 1%) to the solution did not alter the rotation, produced no decrease in its reducing power and did not increase the methoxyl content of the isolated solid to any appreciable extent. This was probably due to the attainment of equilibrium in the reversible reaction:-

Chitose + Methyl alcohol $\rightleftharpoons$ Methyl chitoside + w

From the fact that condensation takes place even in neutral solution with the production of what is probably methyl chitoside it was considered likely that the reverse reaction, namely the hydrolysis of methyl chitoside to chitosi and methyl alcohol, would also be readily effected in slightly acid or neutral solution. This was borne out by the fact that whereas the product, isolated from the chitose solution in methyl alcohol containing 1% acetic acid by making alkaline with a little anhydrous sodium carbonate and evaporating to a dry solid, had a methoxyl content of 10.5%, the same solution on evaporation without neturalisation with sodium carbonate gave a product having a methoxyl content of only 7.4%.

The above methyl alcoholic chitose solution containing 1% acetic acid was made alkaline with anhydrous sodium carbonate and evaporated *in vacuo* to a solid mass which was again taken up in dry methyl alcohol acidified by the addition of glacial acetic acid and allowed to remain at room temperature for 24 hours. Isolated as before the solid amorphous product obtained from the solution was found to have a methoxyl content of 12.7%. No further marked increase in methoxyl content was obtained however when the treatment with dry methyl alcohol was again repeated.

Comparing the methoxyl content of the product thus isolated (12.7%) with the theoretical value for methyl chitoside (17.6%) it was concluded that about 70% of the sugar was present as methyl chitoside. The remainder was probably made up of a small fraction of free chitose, accounting for the slight reducing properties of the material chitose decomposition products and small quantities of sodium acetate and sodium carbonate. The material was practically insoluble in ethyl acetate and acetone and therefore contained no appreciable quantity of chitose dimethylacetal.

Methylation of 'methyl chitoside'.

Several attempts at further methylation of the above product by the method of Haworth resulted in extensive

decomposition and from the very small yields of dark syrupy products no constant boiling fraction distilling under high vacuum was obtained.

Methylation by the Purdie method was also tried. The methylating mixtures were quite alkaline in reaction owing to the presence of small amounts of sodium carbonate and sodium acetate in the crude material so that hydrolysis of methyl chitoside in the process should have been reduced to a minimum. From 10 g. of the amorphous methyl chitoside was thus obtained 2.1 g. of a colourless mobile liquid distilling at 105°C./0.10 mm. and no other constant boiling fraction was isolated.

The product was soluble in water and did not reduce Fehling's solution. After hydrolysis, by heating in 1% hydrochloric acid solution, it still did not reduce Fehling's solution to any appreciable extent. The substance reacted to N/10 caustic soda solution as the ester of an organic acid and it was considered likely to be the methyl ester of trimethyl chitonic acid. The properties of the syrup were further examined and their comparison with those of trimethyl methyl chitonic ester is shown in the following table:-

	Trimethyl methyl chitonic ester	Product from Purdie methylation of 'methyl chitoside'.
Methoxyl content	53.0%	53.3%
Equivalent weight	234	295
Rotation in MeOH	+55.4°	+44.4°
Methoxyl content of the free acid.	42.3%	44.3%

From these figures it was obvious that if the syrup under examination was trimethyl methyl chitonic ester it contained a considerable amount of impurity. It became therefore a matter of importance to prepare a pure crystalli derivative of trimethyl chitonic acid by means of which it could be definitely identified.

The free acid of the ester prepared in the above Purdie methylation was obtained as a viscous syrup distilling at 125-130°C./0.08 mm. Only syrupy or amorphous products were obtained in attempts to prepare a crystalline calcium salt, sodium salt, phenylhydrazide, p-tolylhydrazide, p-nitrophenylhydrazide, semicarbazide and anilide of this acid.

Crystalline silver salt of trimethyl chitonic acid.

A silver salt was prepared by shaking an aqueous solution of the acid with silver carbonate until it was neutral to litmus, filtering and evaporating the clear

filtrate *in vacuo* to dryness at 35-40°C. The resulting white solid mass which was seen under the microscope to consist of fine crystalline needles was extremely soluble in water but was recrystallised by heating to 60°C. with dry methyl alcohol, filtering the hot solution through a layer of charcoal and allowing the liquid to cool. The salt separated from solution in clusters of fine white crystalline needles which were again purified by recrystallisation from methyl alcohol and dried in a vacuum desiccator placed in the dark. Analysis figures were in agreement with the theoretical values for the silver salt of trimethyl chitonic acid.

	C	H	Ag	$.OCH_3$
Values found by analysis.	33.00%	4.90%	33.33%	28.3%
Theoretical for silver trimethyl chitonate.	33.03%	4.59%	33.03%	28.4%

The silver salt showed signs of decomposition on exposure to light for any length of time. Its aqueous solution had a rotation of +43.30°.

From the trimethyl chitonic acid, previously prepared by methylation of chitonic acid, was obtained a crystalline silver salt identical with the above as is apparent from its rotation in aqueous solution (+43.24°) and the following analysis figures:-

	C	H	Ag	.OOH3
Values found by analysis.	33.51%	5.28%	35.18%	28.1%
Theoretical for silver trimethyl chitonate.	33.03%	4.59%	33.03%	28.4%

Trimethyl chitonic acid (methyl ester) is therefore the main product isolated in the Purdie methylation of the material, suspected to consist mainly of methyl chitoside.

Attempted preparation of β-methyl chitoside.

The methyl chitoside prepared as described above is probably the α-isomer since, as a general rule, the α-methyl hexoside is the predominant product of condensation formed in an acid methyl alcoholic solution of the hexose. In the hope that the β-methyl chitoside would prove to be a much more stable derivative attempts were made to prepare it by a method indicated by Haworth and Leitch (J.C.S., 113, 194) who showed that the action of caustic soda on an acid solutio of glucose containing excess dimethyl sulphate resulted in the formation of β-methyl glucoside. The product formed by a similar treatment of chitose was an amorphous solid of methoxyl content 12-13% but this too proved to be extremely unstable and neither by the Purdie nor the Haworth method of methylation was any dimethyl methyl chitoside obtained.

Chitose dimethylacetal.

Pursuing the study of chitose condensation with methyl alcohol, a volume of the solution (30 g. of dry chitose in 400 ml. of dry methyl alcohol) was acidified with dry hydrochloric acid gas to an extent of 0.25%. A slight fall in rotation of the solution at room temperature was observed over a period of 5 days after which it remained unaltered. From the syrup obtained by neutralising and evaporating the solution at 30-35°C. in vacuo only 9.1 g. of an ethyl acetate soluble fraction was extracted. Expecting to obtain a further but smaller quantity of the ethyl acetate soluble material from the residual syrup it was taken up in 200 ml. of dry methyl alcohol containing 0.5% hydrochloric acid and the solution again left at room temperature for 5 days. The unexpectedly large quantity, amounting to 12.0 g., of an ethyl acetate soluble syrup prepared from this residue indicated that, in the previous case where the reaction had been carried out in 0.25% methyl alcoholic hydrochloric acid, the condensation had been interrupted long before it had attained the state of equilibrium represented approximately thus:

Chitose ⇌ Chitose dimethylacetal.

Such being the case the observed rotational change in the solution could give no indication of the extent to which chitose condensation had occurred and it was probably due to a condensation of methyl alcohol with some chitose

decomposition product.

From the combined ethyl acetate soluble fractions (20.1 g.) repeated extraction with ether removed 1.3 g. of material obtained as a light yellow viscous syrup on evaporation of the ether extract.

In previous experiments it was shown that the chitose dimethylacetal formed by the action of methyl alcoholic hydrochloric acid on chitose was practically all contained in the ethyl acetate soluble, ether insoluble fraction and in this case it was expected that the material would be a much purer sample of chitose dimethylacetal than that previously obtained from a specimen of chitose containing a high percentage of decomposition products. This was borne out by the results of analysis which compared much more favourably with the theoretical values for chitose dimethylacetal than did the analysis of the sample previously prepare

	C	H	OCH_3
Values obtained by analysis.	47.16%	7.92%	28.7%
Theoretical for chitose dimethylacetal.	46.15%	7.69%	29.8%
Analysis of an earlier sample prepared from the cruder form of chitose.	47.64%	7.66%	24.3%

This chitose dimethylacetal syrup was quite soluble in water and the aqueous solution had a constant rotation of $+30.95^{\circ}$.

Methylation of the relatively pure sample of chitose dimethylacetal.

Trimethyl chitose dimethylacetal.

Methylation of 10 g. of the purer chitose dimethylacetal syrup by Haworth's method resulted in the formation of 6.1 g. of a colourless mobile liquid which distilled at 90-95°C./0.08 mm. It had a methoxyl content of 60.1%. A further methylation of this product by the method of Purdie gave 6.0 g. of a similar syrup boiling at 90-92°C./0.08 mm. It had a constant rotation in neutral aqueous solution of +39.77° and gave the following analysis figures:-

	C.	H.	$.OCH_3$
Values found by analysis.	53.00%	8.73%	61.1%
Theoretical for trimethyl chitose dimethylacetal.	52.80%	8.80%	62.0%
Values from the fully methylated chitose previously prepared.	53.40%	8.50%	56.7%

Hydrolysis of trimethyl chitose dimethylacetal.

When a quantity of trimethyl chitose dimethylacetal was dissolved in a 1% solution of hydrochloric acid a small decrease in rotation was observed but after 7 days at room temperature a test portion gave only a very faint Schiff's

reaction and on evaporation with semicarbazide no crystalline trimethyl aldehydochitose semicarbazone separated from the syrupy product. Suspecting from this that hydrolysis had proceeded to only a negligible extent the amount of acid in the solution was increased to a concentration of 5% but over a further period of 7 days at room temperature no alteration in rotation was detected. In this case however a neutralised portion of the solution rapidly restored the colour to Schiff reagent and on evaporation with semicarbazide a 60% yield of trimethyl aldehydochitose semicarbazone separated in crystalline form.

Attempted degradation of trimethyl chitose dimethylacetal and trimethyl chitonic acid.

Having confirmed the fact that the condensation product formed from chitose in methyl alcoholic hydrochloric acid is chitose dimethylacetal, that methylation of the latter produces trimethylchitose dimethylacetal and that chitose is undoubtedly an anhydrohexose, further evidence was sought for the hypothesis that the anhydro ring exists in the 2:5 position. It was hoped that this object might be achieved by degradation of trimethylchitose dimethylacetal or trimethyl chitonic acid and isolation of a methylated product of known constitution.

After prolonged treatment of these derivatives with concentrated nitric acid (d. 1.42) at 90°C., in both cases only trimethyl chitonic acid, isolated as the crystalline silver salt, was found in the reaction product.

Trimethyl chitonic acid was unaffected by Fenton's reagent.

Oxidation of trimethyl chitonic acid with alkaline permanganate proceeded only very slowly and a study of the rate and extent of decomposition (Graph 8) revealed the fact that almost complete destruction of the molecule was involved and that at no stage in the reaction could one expect to isolate a comparatively stable intermediate product.

It was considered possible that by subjecting the amide of trimethylchitonic acid to the Hoffmann degradation a

derivative of 2:3:5trimethyl d-arabinose might be obtained but again only unchanged trimethylchitonic acid was recovered from the reaction product. It is noteworthy that, during the course of this experiment in the preliminary conversion of trimethylchitonic acid to the amide, the latter was obtain in crystalline form. Purified by recrystallisation from acetone it separated as a mass of white crystalline needles which melted at 115-116°C. It had a constant rotation in aqueous solution of +47.6°. Microanalysis carried out on this compound has given a wide and unsatisfactory range of figures for carbon, hydrogen, nitrogen and methoxyl content. Nucleation of the syrup obtained six years previously in an attempt to prepare this crystalline amide resulted in a rapid crystallisation of the mass.

Finally, in view of the fact that chitose is converted to d-glucosazone by digestion with phenylhydrazine in acetic acid solution, it seemed reasonable to suppose that trimethyl aldehydochitose on similar treatment would be converted to a trimethyl d-glucosazone which, if chitose has a 2:5anhydro structure, would be the known crystalline 3:4:6trimethyl d-glucosazone. Only an oily product, however, was obtained from the reaction and attempts at crystallising it from a large number of solvents were unsuccessful.

The structure and configuration of chitose.

The following review of the more important results of this work and of the previous history of chitose establishes almost with certainty the fact that chitose is 2:5anhydromannose.

Analyses of the crystalline hydrazones and methylated derivatives of chitose has shown beyond doubt that it is an anhydrohexose.

Its method of preparation and its conversion to d-glucosazone indicates that it is either an anhydromannose or an anhydroglucose.

That the anhydro ring of chitose is situated in the same position as that of chitonic acid, shown by Fischer and Andraea to be a 2:5 anhydrohexonic acid, has been confirmed by the preparation of trimethylchitonic acid indicated as follows:-

Chitonic acid. —Methylation→ Trimethylchitonic acid

Trimethylchitonic acid ←Hydrolysis and oxidation— Trimethylchitose dimethylacetal

Chitose. —Methylation→ Trimethylchitose dimethylacetal

Chitose is therefore either 2:5anhydroglucose or 2:5anhydromannose.

The reducing properties of chitose together with the fact that it fails to restore the colour to Schiff's reagent

have indicated the presence of a glucosidic ring structure in the molecule. From a study of molecular models of 2:5anhydroglucose and 2:5anhydromannose it is plain that a glucosidic ring structure could be formed with far less intramolecular strain in the case of 2:5anhydromannose (in the 1:4 position) that in the case of 2:5 anhydroglucose. These observations, together with the work of Levene and his report that epichitose does exist as a free aldehyde, confirm the hypothesis of this worker that chitose is 2:5anhydro-mannose.

EXPERIMENTAL.

PREPARATION and PROPERTIES OF CHITOSE SOLUTION.

Preparation of chitose solution.

Following the directions of Zechmeister and Toth (Ber., 66B, 552) 7 g. of silver nitrite (2 mols.) were added to a cold solution of 5 g. of glucosamine hydrochlorid in 35 ml. of distilled water and the mixture placed in the refrigerator for one hour. Every few minutes the solid material was ground with a glass rod to prevent the silver nitrite being coated with a layer of silver chloride. The solution was then left in the dark at room teperature for 24 hours. After filtering with suction through a Buchner funnel the clear greenish yellow solution was acidified with 3 ml. of 2N hydrochloric acid and again filtered through a fresh filter paper. The liquid was then strongly aerated for 15 minutes to remove the excess nitrous acid.

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d-Arabinosazone from chitose solution.

To the above cold solution of chitose was added a few crystals of sodium acetate, 1 ml. of 50% acetic acid and 10 ml. of phenylhydrazine. On standing at room temperature there rapidly separated from this solution a small quantity of a yellow crystalline compound resembling d-glucosazone wh examined under the microscope. After 1 hour this product we removed by filtration and left to dry at room temperature.

Weight of the crude product ... ... 0.09 g.

Melting point ... ... 135-142°C.

The substance was recrystallised from hot water, filtered, washed with a little acetone and ether and dried in air on a porous tile.

Melting point of the purified product ... 158°C.

" " " specimen of d-arabinosazone. 158-159°C

Mixed melting point ... ... 158°C.

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d-Glucosazone from chitose solution.

The mother liquor obtained after the separation of arabinosazone in the preceding experiment was heated in a boiling waterbath for 2 hours, during which period a large crop of a yellow crystalline material separated. After cooling, the solid material was removed by filtration and recrystallised from 96% alcohol. It was left to dry in air at room temperature.

Weight of purified product ... ... 1.10 g.

Melting point ... ... 200°C.

" " of specimen of d-glucosazone. 205°C.

Mixed melting point ... ... 200-202°C.

Both samples were dissolved in hot 96% alcohol, the solutions treated with a little charcoal, filtered and set aside to crystallise. Both of the crystalline products,

separated from the solution by filtration, were washed first with a little absolute alcohol and then ether and left in air to dry.

Melting point of both substances ... ... 208°C.

Mixed melting point ... ... 208°C.

Some properties of chitose solution.

The solution gives an intense Molisch's reaction.

It rapidly reduces Fehling's solution at room temperature

It does not restore the colour to Schiff's reagent.

It gives a strong osone reaction (Ariyama's glyoxal reaction (J. Biol. Chem, 74, XLV) found to be positive for solutions containing osones (Hynd - unpublished result in this laboratory).

It rapidly decolourises permangenate solution in the cold.

Effect of aeration on the production of arabinosazone from chitose solution.

36 ml. of chitose solution were prepared according to the method of Zechmeister and Toth, the final stage of aeration to remove nitrous acid being omitted. The solution was divided into nine 4 ml. volumes and each, after aeration

with the gas indicated in the table, treated as follows:-

The osone reaction was carried out on a small test portion of the solution. To the remainder was added 1 ml. of phenylhydrazine, 0.1 ml. of 30% acetic acid and a crystal of sodium acetate and the solution left at room temperature for 2 hours. The results of these experiments are tabulated below.

Soln.	Gas used in aeration	Time of aeration	Osone reaction	Amount of d-arabinosazone deposited from solution.
(1)	not aerated	--	very feint	none.
(2)	Carbon di-oxide.	5 mts.	" "	slight turbidity.
(3)	" "	20 "	" "	" "
(4)	coal gas	5 "	" "	" "
(5)	" "	20 "	" "	" "
(6)	atmospheric air.	5 "	strong	small cryst. deposit
(7)	" "	10 "	intense	larger " "
(8)	" "	20 "	"	same qty. as from soln (7).
(9)	" "	30 "	"	ditto.

In each case when the filtered solution was heated in a boiling water bath for 2 hours a good yield of crystalline d-glucosezone separated.

Preparation of the 2:4dinitrophenylhydrazones of glucose and mannose.

0.9 g. of glucose was dissolved in 25 ml. of 50% aqueous acetic acid and to the solution was added a hot solution of 1 g. (1 mol.) of 2:4dinitrophenylhydrazine in 50 ml. of glacial acetic acid. The solution was left overnight at room temperature and then evaporated *in vacuo* at 40°C. to a dry reddish yellow crystalline mass. This was digested with 100 ml. of hot water and the filtered solution evaporated at 40°C. under diminished preusure to about 10 ml. After the solution had cooled the crystallised glucose 2:4dinitrophenylhydrazone was filtered off and purified by dissolving in 96% alcohol at 50°C. and adding 5 times the volume of ether to the solution. Light yellow crystalline needles separated when the solution was left at room temperature overnight. The crystalline mass was filtered, washed with a little ether and dried in a vacuum desiccator over concentrated sulphuric acid.

Weight of the purified material ... ... 1.5 g.

Melting point ... ... 119°C.

Mannose 2:4dinitrophenylhydrazone was prepared in exactly the same way.

Melting point of mannose 2:4dinitrophenylhydrazone..176°

Preparation of chitose 2:4dinitrophenylhydrazone.

1 g. of dry amorphous chitose syrup, prepared by evaporation of chitose solution in vacuo at 40°C. to a solid mass, was treated in exactly the same way as glucose in the preceding experiment for the preparation of glucose 2:4dinitrophenylhydrazone. Evaporation of the reaction mixture in vacuo at 40°C. gave a dark red oily product from which a crystalline material separated on standing overnight. The product was digested with 200 ml. of hot water and the orange yellow solution decanted from the insoluble tarry residue. On evaporating the solution at 40°C. under diminishe pressure there first separated a red coloured oil which did not crystallise and then a mass of light yellow crystalline needles. After twice recrystallising from 25% acetic acid the purified crystals were washed with ether and dried over concentrated sulphuric acid in a vacuum desiccator. The compound had a melting point of 192°C. Its solution gave no Molisch's reaction so that it was probably not a carbohydrate derivative. It proved to be identical with a sample of the acetate of 2:4dinitrophenylhydrazine prepared by evaporating a solution of the hydrazine in glacial acetic acid. Melting point of 2:4dinitrophenylhydrazine acetate..192

Mixed melting point of the two specimens 192

A solution of chitose obtained by deamination of 2.5 g. of glucosamine hydrochloride was evaporated in vacuo at 40°C.

to a stiff syrup which, following the directions of Glaser and Zuckermann in their preparation of glucose 2:4dinitrophenylhydrazone (Z. physiol. Chem., 1927, 44), was taken up in solution in 70 ml. of 96% alcohol. To this was added 2.3 g of 2:4dinitrophenylhydrazine and the mixture was refluxed on a boiling water bath for 2 hours. The clear orange red solution thus obtained was evaporated to a viscous syrup in vacuo at 40°C. The oily product which failed to crystalli was digested with 200 ml. of water at 60°C. and the resulting solution was decanted from the remaining insoluble semisolid tarry residue. The aqueous solution was evaporated in vacuo at 40°C. and the red coloured oily residue obtained crystalli on standing overnight. A quantity of the material which fail to crystallise was removed by digesting the mass at room temperature with 2-3 ml. of 96% alcohol and filtering with suction. Thus was isolated an orgaṇe yellow crystalline product which after drying in air weighed 1.8 g.

On dissolving the compound in a number of solvents the solution darkened appreciably on heating and an oily product separated with the solid on cooling. A degree of purificatio was achieved by digesting the material with water at 60°C., treating the solution with a little charcoal, filtering and allowing the clear orange coloured solution to cool. The yellow crystalline product, which was filtered off and allowed to dry in air at room temperature, melted at 172-173°C.

Further recrystallisation by the same method or from 90% alcohol did not result in a product of higher melting point.

From absolute ethyl alcohol or dry methyl alcohol at 60°C. however, a lemon yellow crystalline solid separated on cooling in clusters of needles which after filtering was washed with a little of the fresh solvent and allowed to dry in air at room temperature. The compound thus obtained melted sharply at 175°C. Repeated recrystallisation from dry methyl alcohol did not yield a specimen having a higher melting point.

The fact that considerable decomposition of the hydrazone takes place in hot aqueous solution was demonstrated by dissolving a quantity of the purified substance in hot water and heating in a boiling waterbath. After 5 minutes the colour of the solution had appreciably darkened though on cooling an abundant crop of yellow crystals separated. On heating for a further 30 minutes, however, and then allowing to cool comparatively few crystals separated, most of the material coming out of solution as a dark oily product.

An aqueous solution of the pure compound gave an intense Molisch's reaction indicating that it contained a carbohydrate residue.

Melting point of mannose 2:4dinitrophenylhydrazone ... 176°C.

Melting point of chitose 2:4dinitrophenylhydrazone ... 175°C.

Mixed melting point of the chitose and mannose 2:4dinitrophenylhydrazones 156-160

Rotation: $(d)_D^{19°} = + 49.79°$ in methyl alcohol. c = 0.643%.

Analysis:

	C.	H.	N.
Found	42.22%	4.05%	16.20%
Anhydrohexose derivative requires..	42.11%	4.09%	16.37%
Normal hexose derivative requires..	40.00%	4.44%	15.56%

Yields of chitose 2:4dinitrophenylhydrazone obtained under varying experimental conditions.

5 g. of glucosamine hydrochloride were deaminated by the method of Zechmeister and Toth and the resulting chitose solution evaporated in vacuo at 40°C. to a moderately mobile syrup. To this was added a solution of 4.6 g. of 2:4dinitrophenylhydrazine in 480 ml. of hot 96% alcohol and the mixture was shaken until complete dissolution of the chitose syrup had occurred. The resulting red coloured solution was rapidly cooled, made up to a volume of 500 ml. with alcohol and subsequently divided into four 125 ml. volumes.

Solution 1 was left standing at room temperature.

" 2 " refluxed for 20 mts. on a boiling water bath.

" 3 " " " 1 hour " " " " "

" 4 " " " 2 hours " " " " "

Each solution was evaporated in vacuo at 40°C. to a dark viscous product which was then thoroughly digested at 50°C. with 500 ml. of water. The aqueous solution was decanted

from the insoluble tarry residue and evaporated *in vacuo* at 40°C. to dryness. The water soluble extract was in each case dissolved in 5 ml. of 96% alcohol, nucleated with a few crysta of pure chitose 2:4dinitrophenylhydrazone and left at room temperature for 7 days to crystallise. The crystalline hydrazone was then filtered with suction, washed with 5 ml. of fresh 96% alcohol and left to dry in air at room temperature.

Wt. of crystalline hydrazone from solution 1 .. 1.05 g.
" " " " " " 2 .. 0.98 g.
" " " " " " 3 .. 0.85 g.
" " " " " " 4 .. 0.76 g.

Methylation of chitose 2:4dinitrophenylhydrazone.

To a 25 ml. conical flask was added 10 ml. of dry acetone 2 g. of pure chitose 2:4dinitrophenylhydrazone, 2.1 g. of methyl iodide and 1.75 g. of silver oxide. The flask was fitted to a reflux condenser and immersed in a water bath maintained at a temperature of 35-40°C. The solid hydrazone rapidly passed into solution and after 6 hours the bath temperature was slowly raised to 60°C. The mixture was then cooled and filtered and the clear red solution evaporated *in vacuo* at 40°C. to a dark red oil. The material was subjected to a further 4 methylations without the addition of acetone and the final viscous dark red syrup obtained was

left in the refrigerator for several days. No crystalline material separated however and subsequent attempts to crystall the substance from a number of solvents were unsuccessful. Th product was found to consist of an ether soluble fraction and an ether insoluble fraction present in approximately equal quantity. The latter was also subdivided into methyl alcohol soluble and insoluble fractions. From these facts it seemed that extensive decomposition had attended the methylation process and the attempt was therefore abandoned.

Benzoylation of chitose 2:4dinitrophenylhydrazone.

(1) To 0.34 g. of chitose 2:4dinitrophenylhydrazone in 5 ml. of 10% caustic soda solution was added 0.42 g. of benzoyl chloride. On vigorous shaking the benzoyl compound was precipitated as a yellow almost solid amorphous mass. The derivative was thoroughly kneaded in several changes of distilled water but crystallisation did not take place. Attempts at crystallising the material from a large number of organic solvents were unsuccessful.

(2) 2.0 g. of chitose 2:4dinitrophenylhydrazone were dissolved in an ice cold solution of 10 ml. of pyridine containing 5 g. (6 mols.) of benzoyl chloride and the solution left overnight at room temperature. On then pouring into 100 ml. of cold water a yellow oily product separated which became semi-solid when kneaded thoroughly in several changes of distilled water. The product was taken up in 150 ml. of ether

and the solution cleaned by shaking in a separating funnel three times with 1% aqueous hydrochloric acid solution and then twice with distilled water. The etherial solution was dried by leaving in contact with anhydrous sodium sulphate overnight. It was then filtered and left in a desiccator over concentrated sulphuric acid. No crystalline material separate only a yellow oily product being obtained which became quite solid when evaporation was complete and swelled up to a brittl amorphous mass when the desiccator was evacuated. Attempts at crystallising it from a large number of solvents were not successful.

Acetyl derivatives of chitose 2:4dinitrophenylhydrazone and glucose 2:4dinitrophenylhydrazone.

(1) Glucose 2:4dinitrophenylhydrazone acetyl derivative.

2 g. of pure glucose 2:4dinitophenylhydrazone was added to an ice cold solution of 10 ml. of pyridine containing 4.5 g. of acetic anhydride. The mixture was kept at 0^{o}C. for 2 hours until the solid hydrazone had completely passed into solution. After leaving at room temperature overnight the clear orange red solution was poured into 100 ml. of ice cold water. The yellow oily product which separated and which solidified on kneading was filtered off and left to dry in air at room temperature. Difficulty was experienced in recrystallising the product which separated from most solvents as a stiff gel

in which no crystals could be detected under the microscope. Finally it was obtained as fine yellow crystalline needles by recrystallisation from glacial acetic acid. The product was filtered, washed several times with small volumes of ether and dried in a vacuum desiccator over concentrated sulphuric acid.

Melting point ... ... 96°C.

Chitose 2:4dinitrophenylhydrazone acetyl derivative.

A large test tube containing 4.5 g. of acetic anhydride in 10 ml. of dry pyridine was cooled in a freezing mixture. To this solution was added 2 g. of pure chitose 2:4dinitrophenylhydrazone and the mixture agitated until all the solid material had dissolved (45 mts.) to form a clear orange coloured solution. This was left at 0°C. in the refrigerator for a period of 5 hours then at room temperature overnight. On then pouring into 100 ml. of ice cold water a yellow oil separated. On kneading the product in several changes of distilled water it gradually became more viscous and was finally obtained as a semisolid yellow mass. This was kneaded thoroughly in 3, 50 ml. volumes of a 1% hydrochloric acid solution to remove any remaining pyridine then twice again in distilled water. The material however still retained a strong odour of pyridine and so was dissolved in 150 ml. of ether and the solution thoroughly shaken in a separating funnel

with 2, 100 ml. volumes of 1% hydrochloric acid and three times with fresh distilled water. The etherial solution was left in contact with anhydrous sodium sulphate overnight, filtered, and the clear orange yellow solution placed in a desiccator over concentrated sulphuric acid. As evaporation proceeded a yellow oil separated from solution but there was no sign of crystallisation. The product was dried in a vacuum desiccator over concentrated sulphuric acid and was obtained as a solid lemon yellow amorphous mass. Attempts to crystalli the material from a wide range of solvents were unsuccessful.

Attempted preparation of a chitose ethylidene condensation product.

An aqueous solution of chitose, prepared from 5 g. of glucosamine hydrochloride was evaporated *in vacuo* at 40°C. to dryness. The brittle amorphous mass was reduced to a fine powder by shaking with several pieces of glass rod. To this was added 15 ml. of dry paraldehyde containing 0.05 ml. of concentrated sulphuric acid and the mixture was shaken for 24 hours. The solid material darkened somewhat during the process but none appeared to have dissolved. The mixture was filtered and the residue after washing with a little pure paraldehyde and petroleum ether was dried *in vacuo* at 40°C. The solid readily dissolved in absolute alcohol but no crystalline material separated on evaporating the solution.

A quantity was heated in aqueous solution containing 5% sulphuric acid but no odour of acetaldehyde was detected in the vapour nor did the neutralised solution restore the colour to Schiff's reagent.

The paraldehyde solution filtered from the reaction mixture was neutralised with barium carbonate, filtered and evaporated *in vacuo* at 40°C. but only a negligible quantity of a dark syrup was obtained. Apparently no condensation had occurred.

Attempted condensation of chitose with acetone.

1 g. of dry amorphous chitose and a few glass beads were shaken for 6 hours in a stoppered bottle with 100 ml. of dry acetone containing 4 ml. of concentrated sulphuric acid. The clear solution obtained by filtration was shaken with an excess of barium carbonate until neutral and again filtered. Only an extremely small quantity of a dark syrupy residue was obtained on evaporation of this solution *in vacuo* at 40°C.

Attempted condensation of chitose with benzaldehyde.

Dry amorphous chitose obtained by the deamination of 5 g. of glucosamine hydrochloride was placed in a small stoppered bottle with 10 ml. of redistilled benzaldehyde and 3.5 g. of powdered anhydrous zinc chloride. The mixture

after shaking for 24 hours was extracted with 150 ml. of chloroform which should take up with the excess benzaldehyde any chitose benzylidene derivative formed in the reaction. The chloroform solution was well shaken with 10 g. of anhydrous sodium carbonate, filtered and evaporated *in vacuo* at 40°C. to a syrup. To part of this residue of benzaldehyde containing any chitose benzylidene derivative which might have been formed was added an excess of petroleum ether but no benzylidene compound was thus precipitated. The remainder of the residue was extracted 4 times with 10 ml. volumes of hot water and the combined aqueous extracts evaporated *in vacuo* at 40°C. Only a small quantity of a white solid, identified as benzoic acid was thus obtained.

Acetyl derivative of chitose.

10 g. of dry amorphous chitose were added to an ice cold solution of 40 g. of acetic anhydride in 65 ml. of dry pyridine and the mixture left in the refrigerator overnight during which time most of the solid chitose had passed into solution. Complete dissolution occurred after the mixture had been left for a day at room temperature giving a light reddish brown coloured solution. When this was poured slowly into 500 ml. of cold water kept vigorously stirred, there separated a sticky brown mass which became semisolid after prolonged kneading in fresh distilled water.

The water was decanted off and the product dissolved in 50 ml of ether. This solution was washed twice with 50 ml. of 1% hydrochloric acid solution and then twice with the same volum of distilled water in a separating funnel. After drying in contact with anhydrous sodium sulphate overnight the etherial solution was filtered and evaporated in a desiccator over concentrated sulphuric acid to a brown syrup which did not crystallise. When the desiccator was evacuated the product swelled up and was finally obtained as a light brittle amorphous mass.

Analysis.

	C	H	$.CO.CH_3$
Found.	49.84%	5.48%	56.4%
Pentaacetylhexose derivative requires.	49.23%	5.64%	55.1%
Triacetyl anhydrohexose derivative requires.	50.00%	5.56%	44.8%.

Benzoylation of chitose.

To 10 ml. of chitose solution containing theoretically 0.75 g. of chitose was added 4.0 g. of benzoyl chloride. Following the addition of a few drops of 40% caustic soda solution the mixture was shaken vigorously. The shaking process was interrupted from time to time and further additio of small quantities of alkali were made until the solution remained alkaline to litmus paper. The semisolid amorphous mass which separated was kneaded thoroughly in several

changes of distilled water. Attempts at crystallising the product from a large number of solvents met with no success.

METHYLATION OF CHITOSE.

Attempted deacetylation and methylation of the chitose acetyl derivative.

3.5 g. of the dry chitose acetyl derivative were placed in a methylating flask with 50 ml. of carbon tetrachloride and 50 ml. of water. To the vigorously stirred mixture was added through dropping funnels 50 ml. of dimethyl sulphate and simultaneously at an equivalent rate 120 ml. of 40% caustic soda solution. The addition was at first made very slowly over a period of 24 hours at room temperature about ¼ of the reagents having been added at the end of this stage. The temperature was then raised to 40°C. and the reagents added comparatively rapidly so that the addition was completed in about 4 hours. Stirring of the mixture was continued for one day at room temperature to ensure complete hydrolysis of the dimethyl sulphate. On extraction of the mixture with 2, 50 ml. volumes of chloroform and evaporation of the combined extract in vacuo at 40°C., 0.3 g. of a dark viscous syrup was obtained. Distillation of the product at 0.1 mm. resulted in 0.2 g. of a viscous syrup which distilled gradually between 120° and 180° C. No appreciable quantity of distillate came over at any fixed temperature which indicated that the product was a complex mixture.

Attempted methylation of free chitose.

A solution of chitose prepared by deamination of 40 g. of glucosamine hydrochloride was evaporated *in vacuo* at 40°C. to a mobile syrup. This was methylated by the method which Haworth and Leitch employed in the complete methylation of free glucose (J.C.S. 113, 194) with 109 ml. of dimethyl sulphate and 109 g. of sodium hydroxide in 190 ml. of water. The dark syrup obtained by evaporation of the chloroform extract was distilled in high vacuum, all fractions distilling up to 200°C./0.12 mm. being collected. The total weight of this product was only 1.6 g. indicating that extensive decomposition was involved in the methylating process. Part of small fraction of this distillate coming over at 145-150°C. crystallised in long colourless needles. The crude solid was separated from most of the adhering syrup on a porous tile and purified by recrystallising from ethyl acetate. Yield of the purified compound was 0.03 g. It was very soluble in water, alcohol and acetone, sparingly soluble in ether and almost completely insoluble in petroleum ether. It had a moderate solubility in hot ethyl acetate from which solution, on cooling, it separated in fine white crystalline needles. An aqueous solution of the substance did not give a positive Molisch's reaction indicating that it was not a chitose derivative.

Melting point ..... 191-192°C.

Analysis:

C.	H.	N.
54.08%	10.14%	6.02%

The fact that it contained nitrogen confirmed the suspicion that it was not a methylated derivative of chitose.

Methyl chitoside.

Attempts at the preparation of the crystalline methyl chitoside described by Neuberg, Wolff and Niemann (Ber. 35, 4009) and repeated according to their directions have not been successful. The solid which separated from the reaction mixture was recrystallised by dissolving in a small volume of hot water, decolourising with a little charcoal and adding to the clear filtered solution 5 times its volume of absolute alcohol. The solid which gradually separated was filtered off and allowed to dry in air at room temperature. On heating the substance darkened and decomposed at 220-230°C. Its aqueous solution gave a positive Fehling's test and negative Molisch's reaction. The addition of sodium nitrite to its aqueous solution resulted in a gas evolution after which the solution gave an intense Molisch's reaction. A drop of silver nitrate solution in an aqueous solution of the substance gave a precipitate of silver chloride. These tests were sufficient to identify the material as glucosamine hydrochloride probably present as a result of incomplete deamination in the preparation of chitose solution.

On heating a solution of chitose in dry methyl alcohol containing 1½% hydrochloric acid it darkened very rapidly indicating extensive decomposition, and to such an extent that it was not possible to follow the course of the reaction polarimetrically beyond an initial fall in rotation from $+6.9^{\circ}$ to $+6.3^{\circ}$.

The extent of desmination of glucosamine hydrochloride in the preparation of chitose by the method of Zechmeister and Toth.

0.4310 g. of pure glucosamine hydrochloride were accurately weighed out and dissolved in distilled water, the solution being made up to 4 ml. This was cooled to 0°C. in the refrigerator and to the solution was added 0.62 g. of silver nitrite (2 mols.). The reaction flask was immediately connected to a Lunge nitrometer and kept for 1 hour in an ice bath at 0°C. It was then left at room temperature for 24 hours during which period the reaction mixture was frequently shaken. At this point gas evolution had practically ceased.

Final volume of nitrogen evolved	36.6 ml. at 20°C. and 770 mm.
Volume of nitrogen at N.T.P.	34.5 ml.
Theoretical volume of nitrogen at N.T.P., from 0.4310 g. of glucosamine hydrochloride ...	44.8 ml.
Percentage deamination.	77.0%.

Extent of deamination of glucosamine hydrochloride with sodium nitrite.

To a solution of 0.4310 g. of glucosamine hydrochloride in 2 ml. of cold water was added 0.138 g. of sodium nitrite (1 mol.) in 2 ml. of cold water and the reaction flask was immediately attached to a nitrometer. After 72 hours at room temperature gas evolution had practically ceased and the volume of nitrogen collected at 19°C. and 773½ mm. was 40.0 ml.

Volume of nitrogen evolved at N.T.P.	38.0 ml.
Theoretical volume of nitrogen at N.T.P. ...	44.8 ml.
Percentage deamination.	84.8%.

To the partially deaminated solution was then added a further 0.035 g. of sodium nitrite (¼ mol.). Nitrogen evolution again commenced and after 72 hours when it had practically ceased the total volume of nitrogen liberated at 15°C. and 753½ mm. was 45.6 ml.

Volume of nitrogen at N.T.P.	43.6 ml.
Extent of deamination.	97.3%.

Approximate comparison of the chitose content of a solution of glucosamine hydrochloride deaminated by sodium nitrite with that deaminated by silver nitrite.

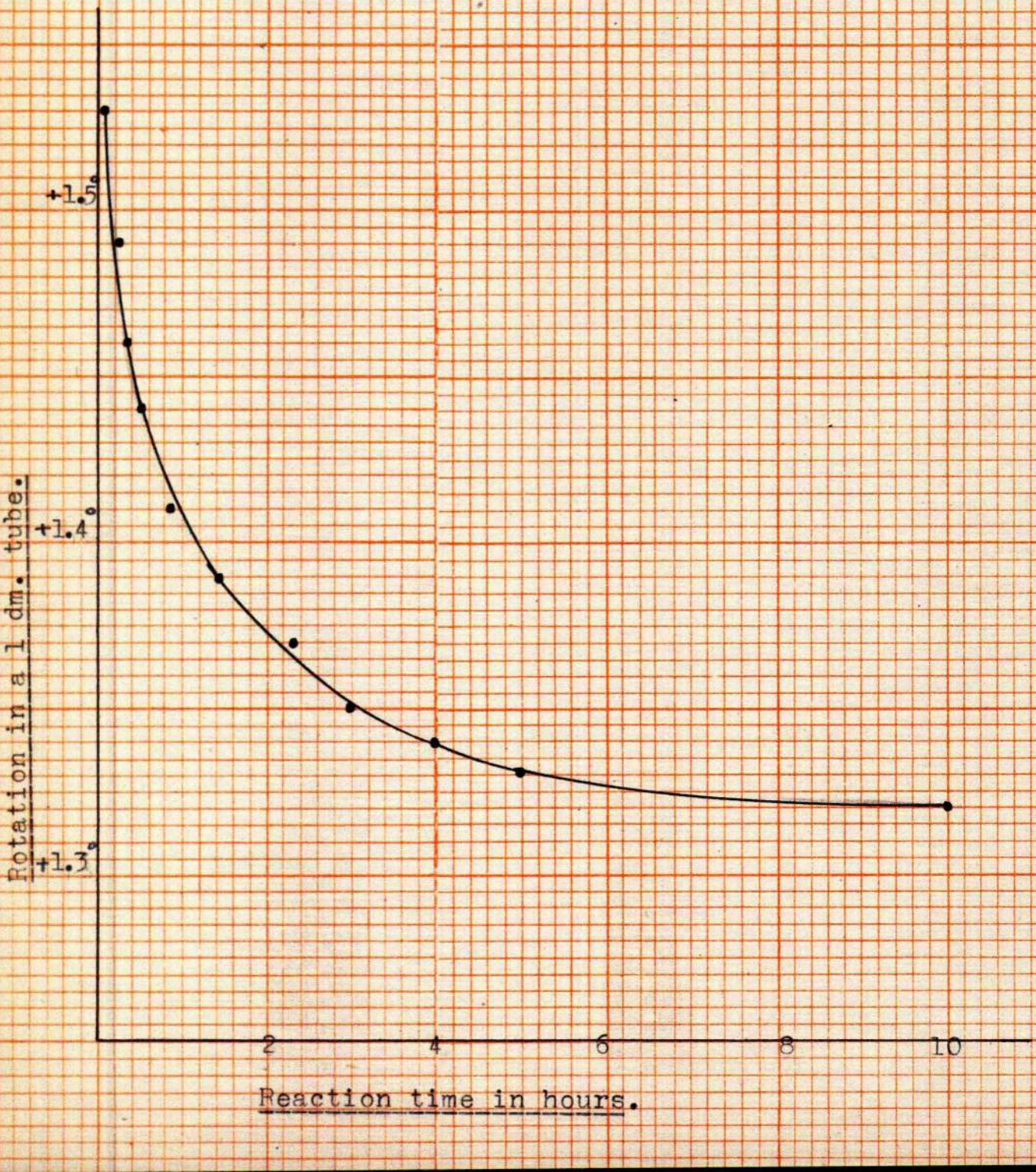
To 25 ml. of a cold aqueous solution containing 2.5 g. of glucosamine hydrochloride was added 1.0 g. (1½ mols.) of sodium nitrite. This was left to deaminate at room temperature for 6 days. To free the solution from excess nitrite it was strongly aerated for 15 minutes after the addition of 1.5 ml. of 2N hydrochloric acid. The resulting greenish yellow solution was evaporated at 40°C. in vacuo to a fairly mobile syrup which was digested with 10 ml. of 96% alcohol thus taking chitose into solution and leaving undissolved most of the crystalline sodium chloride. After filtration the clear solution was made up to 70 ml. with 96% alcohol and treated in exactly the same way as described for the preparation of chitose 2:4dinitrophenylhydrazone (page 80).

Weight of the crude product obtained .... 1.9 g.
Melting point of the purified product .... 175°C.
Mixed m.p. with chitose 2:4dinitrophenylhydrazone ... 175°C.

Weight of the crude hydrazone from chitose prepared by deamination of 2.5 g. of glucosamine hydrochloride with silver nitrite was 1.8 g. From this it would appear that approximately the same yield of chitose is obtained by both methods of deamination.

GRAPH 1.

Changes in rotation of a chitose solution in dry methyl alcohol containing 1% hydrochloric acid.



Attempted preparation of methyl chitoside from chitose obtain
by deamination of glucosamine hydrochloride with sodium nitri

A solution of chitose, prepared from 5.0 g. of glucosamine hydrochloride as described in the preceding experiment was evaporated *in vacuo* at 40°C. to a dry yellow solid. The chitose was dissolved by shaking for a short time with 20 ml. of dry methyl alcohol and the mixture left in a stoppered flask overnight in the refrigerator to allow as much sodium chloride as possible to crystallise out. The yellow methyl alcoholic chitose solution was then filtered and made up to 70 ml. with pure dry methyl alcohol and to this solution was added 5 ml. of methyl alcohol containing 0.75 g. of dried hydrochloric acid gas. The solution thus contained 5% of chitose (theoretical quantity from 5.0 g. of glucosamine hydrochloride) and 1% hydrochloric acid. The solution was kept in a stoppered flask at room temperature, the rate and extent of the observed fall in rotation being indicated by the following table and Graph 1.

Time in hours.	Rotation in 1 dm. tube.
0.05	+1.53°
0.15	+1.49°
0.20	+1.46°
0.30	+1.44°
0.50	+1.41°
1.25	+1.39°
2.20	+1.37°
3.00	+1.35°
5.00	+1.33°
10.00	+1.32°
24.00	+1.31°
36.00	+1.31°

In this case the fall in rotation was not accompanied by a separation of glucosamine hydrochloride nor was there any appreciable darkening in the colour of the solution. The cold solution which remained unchanged in rotation over a further period of 6 days, was then neutralised by shaking with 5 g. of silver carbonate, filtered and the light yellow liquid evaporated in vacuo at 40°C. to a viscous syrup. Even after a period of several months in the refrigerator no crystalline material separated from a sample of this product.

An aqueous solution of the syrup did not reduce Fehling's solution to any appreciable extent contrasting with the powerful reducing action of chitose and providing strong evidence that methylation of the free glucosidic hydroxyl group of the chitose molecule had been effected.

Methylation of methyl chitoside syrup by Haworth's method.

To 27 g. of "methyl chitoside" syrup, prepared as described in the previous experiment, was added just sufficient water to make the syrup mobile and this was methylated by the Haworth process with 100 ml. of dimethyl sulphate and 300 ml. of 30% caustic soda solution. After 3 hours at 65-70°C. when addition of the reagents had been completed the temperature of the reaction mixture was raised to 100°C. for half an hour. After cooling, the mixture was extracted three times with 30 ml. volumes of chloroform and the combined extract left overnight in contact with anhydrous

sodium sulphate. The solution was then filtered and evaporated to a dark mobile syrup in vacuo at 40°C. On distilling the product in high vacuum and collecting all fractions coming over up to 200°C. 17.8 g. of a light yellow mobile product was obtained. By fractional distillation of 34.5 g. of this material obtained from 2 methylations the following fractions were isolated:

Fraction	(1)	...	21.4 g.	distilling at	95-110°C./0.1 mm.
"	(2)	...	1.5 g.	" "	110-120°C. "
"	(3)	...	1.4 g.	" "	120-130°C. "
"	(4)	...	2.0 g.	" "	130-140°C. "
"	(5)	...	1.9 g.	" "	140-150°C. "
"	(6)	...	0.5 g.	" "	150-165°C. "
"	(7)	...	3.0 g.	" "	165-180°C. "
"	(8)	...	2.5 g.	" "	180-200°C. "

In none of these fractions did crystallisation take place.

The first fraction containing by far the largest quantity of material, was again distilled at 0.1 mm. pressure and the large proportion boiling at 100°C. separated.

Weight of material distilling	at	95-100°C. ...	1.7 g.
" " "	"	100°C. ...	14.4 g.
" " "	"	100-110°C. ...	5.0 g.

The material distilling at 100°C./0.10 mm. was considered to be fully methylated chitose.

Analysis: found ... C, 53.40%. H, 8.87%. OCH_3 56.7

Molecular weight : 218.

Formula calculated from the above data : $C_{10}H_{19}O_5$.

Rotation ... $(\alpha)_D^{20^\circ}$ = +28.20° in methyl alcohol. c=2.341%.

Methylation of methyl chitoside syrup by Purdie's method.

7 g. of methyl chitoside syrup were dissolved in dry acetone (5 ml.) and to the solution was added 11 g. of methyl iodide. The reaction flask was fitted to a reflux condenser and immersed in a waterbath at 35-40°C. 5.5 g. of silver oxide were then added in approximately 1 g. lots, each successive addition being made when the reaction had subsided. The methylation process was repeated 6 times to ensure that the product was fully methylated and the final mobile syrup distilled in high vacuum. After the third methylation it was not found necessary to add acetone as the product was quite soluble in methyl iodide. From the product was obtained 3.0 g. of a colourless mobile syrup distilling at 100°C./0.10 mm. This was shown to be identical with the Haworth methylation product as it had a methoxyl content of 56.8% and a rotation in methyl alcohol of +28.44°.

The mobile fully methylated chitose syrup was quite soluble in water, alcohol, ether, chloroform and benzene. Its aqueous solution gave an intense Molisch's reaction and

did not reduce Fehling's solution. A drop of bromine water or permanganate solution was immediately decolourised when added to an aqueous solution of the syrup. After heating a solution of fully methylated chitose in 1% hydrochloric acid for a few minutes it reduced Fehling's solution strongly

STRUCTURE OF FULLY METHYLATED CHITOSE.

Hydrolysis of fully methylated chitose.

5.2 g. of the methylated chitose syrup was dissolved in 100 ml. of N/100 aqueous hydrochloric acid and the solution placed in an incubator at 37°C. There was a gradual fall in the rotatory power of the solution as indicated in the following table but the solution soon became so cloudy with the formation of a fine precipitate the measurement of rotation became impossible.

Time in hours.	Rotation in 1 dm. tube.
0.05	+1.35°
0.20	+1.33°
0.50	+1.30°
2.00	+1.28°

The hydrolysis was therefore carried out as for γ-methyl glucosides, by adding 1 ml. of concentrated hydrochloric acid to make the strength up to approximately N/10 and heating the solution to 100°C. for 2 hours. After cooling, the solution was neutralised by shaking with an excess of silver carbonate. The clear filtered solution readily reduced Fehling's solution and rapidly restored the colour to Schiff's reagent. It was evaporated *in vacuo* to a semi mobile syrup which was distilled in high vacuum, the bulk of the product coming over at 120°C./0.10 mm. (2.5 g.
An aqueous solution of this purified fraction also rapidly restored the colour to Schiff's reagent indicating that it

was a free aldehyde.

Analysis:	C.	H.	OCH_3
found.	52.18%	7.85%	45.39%
A trimethyl aldehydo-enhydrohexose requires.	52.94%	7.84%	45.59%

The syrup has been provisionally named trimethyl aldehydo-chitose.

Attempted preparation of a crystalline derivative of trimethyl aldehydochitose.

(1) Phenylhydrazone.

0.5 g. of trimethyl aldehydochitose syrup were dissolved in 3 ml. of water containing 0.25 g. of phenylhydrazine (1 mo and a few drops of glacial acetic acid. After standing for a short time at room temperature the solution became quite turbid and in a few hours a light red viscous oil had separated. The product was kneaded in 1% acetic acid solution but did not crystallise. Attempts at crystallisatio from a large number of solvents met with no success.

---

(2) 2:4dinitrophenylhydrazone.

0.5 g. of 2:4dinitrophenylhydrazine (1 mol.) was added to a solution of 0.5 g. of trimethyl aldehydochitose in 20 ml. of absolute alcohol and the mixture heated on a boilin

waterbath until dissolution was complete. On evaporation of the clear red solution *in vacuo* at 40°C. a dark red viscous syrup was obtained. Again it was not found possible to isolate the product in crystalline form.

(3) Semicarbazone.

To an aqueous alcoholic solution of semicarbazide formed by adding 0.66 g. of hydrated sodium acetate in 5 ml. of hot 96% alcohol to 0.55 g. of semicarbazide hydrochloride in 5 ml. of water, was added 1 g. (1 mol.) of trimethyl aldehydochitose. On leaving the solution to evaporate spontaneously in air at room temperature, long white crystalline needles separated from solution. The weight of the crude product removed by filtration and dried in air was 1.1 g. The material was twice recrystallised from methyl alcohol and dried in a desiccator over concentrated sulphuric acid.

Melting point .... 148°C.

Rotation ... $(\alpha)_D^{20^\circ} = +37.52^\circ$ in methyl alcohol. c=1.013%.

Analysis:	C.	H.	N.	OCH_3.
found.	46.15%	7.32%	15.90%	35.4%
Trimethyl aldehydochitose semicarbazone requires.	45.98%	7.28%	16.09%	35.6%.

No other crystalline semicarbazone was isolated by working

up the mother liquors in this preparation.

The crystalline semicarbazone was moderately soluble in water and sparingly soluble in ether. Its aqueous solution gave an intense Molisch's reaction.

This derivative was prepared from the fully methylated chitose formed by the Haworth methylation of methyl chitoside syrup. From the methylated chitose formed in the Purdie methylation of methyl chitoside the same crystalline semicarbazone was derived in exactly the same way.

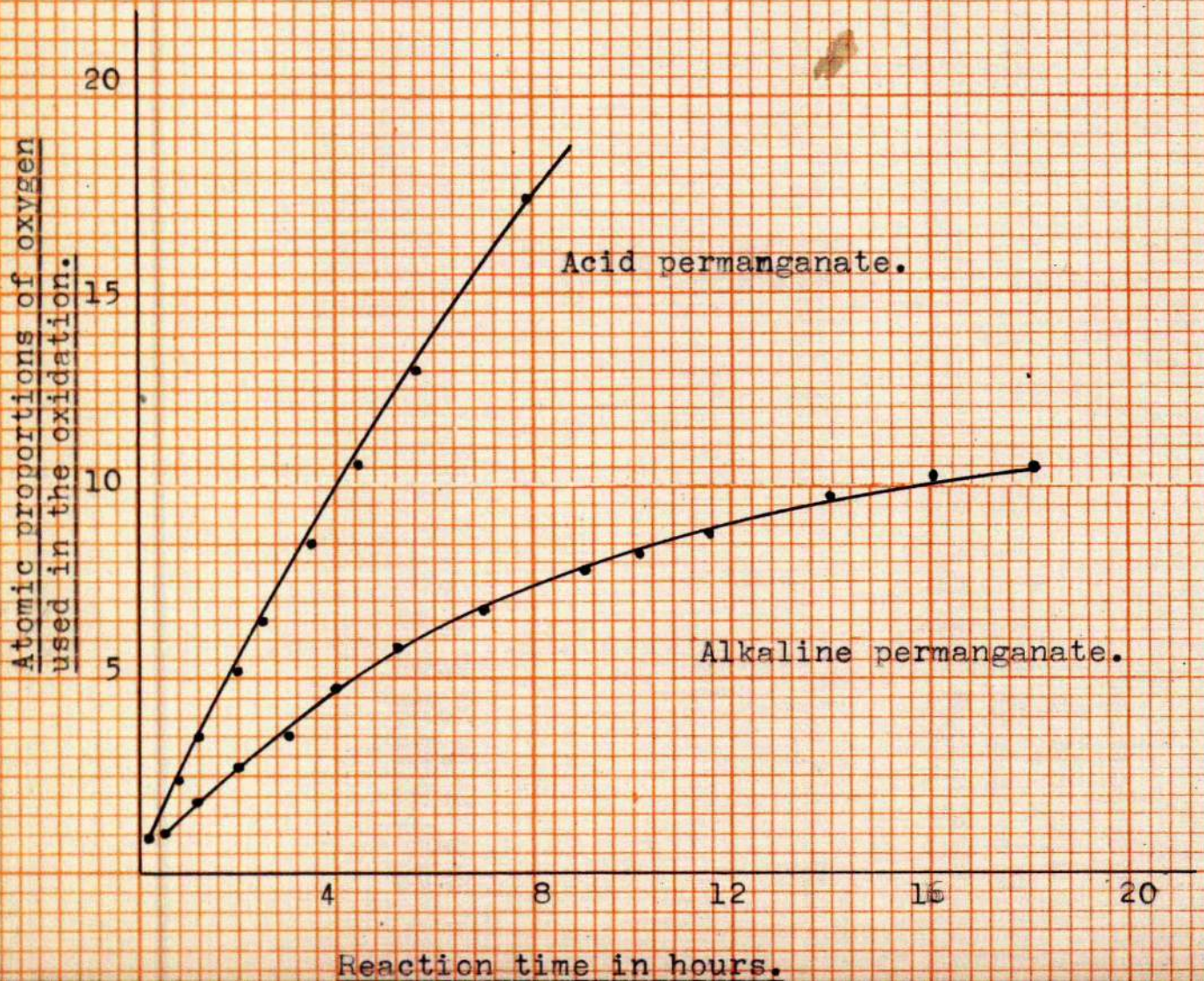
Oxidation of fully methylated chitose with permanganate.

0.1250 g. of the fully methylated chitose syrup were dissolved in 10 ml. of distilled water and a solution of N/20 potassium permanganate run in dropwise from a burette. The first 3.0 - 3.2 ml. of permanganate added were decolourised instantly, after which it became difficult to follow any colour change accurately due to the separation of the dark brown manganese oxide precipitate. A further 0.5 ml. of the permanganate was then added and the solution filtered rapidly through a Buchner funnel. The clear filtrate had the characteristic permanganate colour which faded only slowly and which was still apparent after the solution had been set aside for 20 minutes.

Estimated volume of permanganate (containing 1 atomic proportion of oxygen) equivalent to 0.1250 g. of the fully methylated chitose ... ... 22.9 ml

GRAPH 2.

Oxidation of fully methylated chitose by acid and alkaline permanganate.



Volume of permanganate instantly decolourised ... 3.2 ml.

i.e., approximately 1/7 of the estimated quantity.

Oxidation of fully methylated chitose by alkaline permangenat

50 ml. of N/10 potassium permanganate solution in which was dissolved 0.4 g. of sodium hydroxide was added to a solution of 0.0434 g. of fully methylated chitose in 40 ml. of water and this was made up to 100 ml. in a graduated flask. This was heated to 60°C. in a waterbath and from time to time 5 ml. of the solution were withdrawn, acidified with an excess of 20% sulphuric acid and titrated with a standard solution of N/20 ferrous ammonium sulphate to determine the amount of permanganate used up in the oxidation.

Initially 5 ml. of the solution contained 0.0022 g. of fully methylated chitose syrup and approximately 12.5 atomic proportions of oxygen present as permanganate. The results of this experiment are indicated in the following table and Graph 2.

Reaction time.	Atomic proportions of oxygen used in the oxidation.
5 mts.	0.50
10 mts.	0.65
30 "	1.00
1 hr. 15 "	1.90
2 hrs 0 "	2.75
3 " 0 "	3.85
4 " 5 "	4.75
5 " 20 "	5.70

Reaction time.	Atomic proportions of oxygen used in the oxidation.
7 hrs. 0 mts.	6.75
9 " 0 "	7.75
10 " 15 "	8.25
11 " 30 "	8.75
14 " 0 "	9.75
16 " 0 "	10.25
18 " 0 "	10.50

Oxidation of fully methylated chitose with acid permanganate.

To a solution of 0.0251 g. of the fully methylated chitose syrup in 40 ml. of water was added 50 ml. of N/10 potassium permanganate and 0.5 g. of concentrated sulphuric acid, and the total volume made up to 100 ml. with water. The reaction flask was immersed in a waterbath at 60°C. and at noted intervals of time 5 ml. were removed and titrated with N/20 ferrous ammonium sulphate thus indicating the extent of oxidation.

The results of the experiment are tabulated and recorded graphically as indicated (Graph 2).

Reaction time.	Atomic proportions of oxygen used in the oxidation.
18 mts.	0.08
50 "	2.4
1 hr. 20 "	3.5
2 hrs. 0 "	5.2
2 " 35 "	6.5
3 " 30 "	8.5
4 " 25 "	10.6
5 " 40 "	13.0
7 " 50 "	17.4

It seemed likely from the results of investigation of the oxidation of the syrup with acid or alkaline permanganate that at no stage could one expect the formation of an intermediate oxidation product stable to permanganate which might give some clue to the structure of the fully methylated chitose molecule.

Estimation of the possible unsaturated linkage in the fully methylated chitose molecule.

(1) Wij's method.

0.9663 g. of fully methylated chitose were dissolved in pure carbon tetrachloride and the solution made up to 15 ml. in a graduated flask. 5 ml. of this solution was pipetted into each of two clean dry 300 ml. ground glass stoppered

bottles and into a third similar bottle was transferred 5 ml. of pure carbon tetrachloride. These three tightly stoppered bottles were placed in a dark cupboard at room temprature and, after being allowed to stand for 2 hours, 1 g. of solid potassium iodide and 100 ml. of distilled water were added to each. The solutions were titrated with N/10 sodium thiosulphate using starch indicator solution to determine the end point.

Solution (a) ≡ 22.3 ml. of N/10 thiosulphate solution.
" (b) ≡ 22.5 ml. " " " "
Blank solution ≡ 24.3 ml. " " " "

Wt. of fully methylate chitose in solutions (a) and (b) ≡ .3221 g.

0.3221 g. syrup ≡ 1.90 ml. of N/10 thiosulphate solution.

1.9 ml. of N/10 thiosulphate ≡ 0.0244 g. of iodine.

0.3221 g. fully methylated chitose ≡ 0.023$\overset{44}{5}$ g. of iodine.

i.e., 218 g. " " " ≡ 16.5 g. " "

If fully methylated chitose had the proposed structure it (218 g.) it would absorb 254 g. of iodine, and since only about 1/16th of this quantity of iodine was actually absorbed the postulated unsaturated structure for the compound seems unlikely.

(2) The bromine addition method.

1.0829 g. of fully methylated chitose were dissolved in pure carbon tetrachloride and the solution made up to 15 ml. in a graduated flask. 10 ml. of this solution containing 0.7219 g. of the chitose derivative were transferred to a clean dry 500 ml. glass stoppered bottle and 40 ml. of a standard solution of bromine in pure carbon tetrachloride was added. After standing at room temperature in the dark overnight the bottle was cooled in an ice bath and 100 ml. of ice cold water and 3 g. of potassium iodide were added. The mixture was shaken vigorously until all the potassium iodide had dissolved and then immediately titrated with N/10 sodium thiosulphate solution.

40 ml. standard bromine solution.	≡	128.0 ml. of N/10 thiosulphate.
Titration of reaction mixture.	≡	113.4 ml. " " "
Therefore bromine absorbed	≡	14.6 ml. " " "
	=	0.1172 g. of bromine.

If the chitose derivative had the proposed unsaturated structure the theoretical absorption should be 0.5298 g. of bromine.

Only 1/4 - 1/5 of the theoretical amount of bromine was aborbe

Attempted preparation of trimethyl aldehydochitose by deamination of 3:4:6trimethyl glucosamine hydrochloride.

(1) By deamination with sodium nitrite.

3:4:6trimethyl glucosamine hydrochloride was prepared according to the directions of T. White (J.C.S. 1940, 443). To a cold solution of 5 g. of 3:4:6trimethyl glucosamine hydrochloride in 25 ml. of water was added 1.7 g. (1½ mol.) of sodium nitrite and the solution left at room temperature for 7 days when the evolution of nitrogen had apparently ceased. The resulting clear yellow liquid was acidified with 2 ml. of 2N hydrochloric acid and strongly aerated for 15 minutes to remove any nitrous acid. Then it was evaporated in vacuo at 40°C. to a semimobile syrup which was extracted with 30 ml. of ether. Evaporation of the other extract and distillation of the syrupy residue in high vacuum yielded only 0.5 g. of a mobile liquid boiling at 115-125°C./0.10 mm. The presence of an aldehyde in this product was indicated by the fact that it gave an intense red colour with Schiff's reagent. On treatment of this syrup with semicarbazide a crystalline material began to separate from the solution almost immediately. The solution was allowed to evaporate to a small volume and the crystalline material separated by filtration. The substance did not recrystallise well from methyl alcohol as it was too soluble in the cold solution but crystallised well on cooling its solution in hot water. The purified compound was dried in a vacuum desiccator over

concentrated sulphuric acid.

Melting point	166°C.			
Analysis --	C	H	N	OCH_3
found..	48.91%	5.74%	20.90%	14.6%
Molecular weight... ...	201.			
Formula calculated from the above data	$C_8H_{11}N_3O_3$.			

---

(2) Deamination with silver nitrite.

3.8 g. of 3:4:6trimethyl glucosamine hydrochloride were dissolved in 25 ml. of cold water and to the solution was added 4.6 g. (2 mols.) of silver nitrite. The mixture was left at room temperature for 7 days after which nitrogen evolution was no longer apparent. Filtration gave a light yellow coloured solution from which excess silver nitrite was removed by the addition of 2 ml. of 2N hydrochloric acid, filtering off the precipitated silver chloride, and removing the nitrous acid by vigorous aeration for 15 minutes. The solution was then evaporated in vacuo at 40°C. and the syrupy product treated as described in the preceding experiment. 0.2 g. of a crystalline semicarbazone was thus isolated. It melted at 166°C. and no depression of melting point was observed when it was mixed with the compound described above.

Preparation of ω-methoxy-5-methyl furfural semicarbazone.

This compound was prepared from tetramethyl γ-fructose by the method described by Haworth, Hirst and Nicholson (J.C.S., 1927, 1513).

Melting point ... ... 166°C.

Mixed melting point with the semicarbazone prepared from the 3:4:6trimethyl glucosamine hydrochloride deamination product... 166°C.

The two compounds are therefore identical.

The action of 8% hydrochloric acid solution on trimethyl aldehydochitose.

A solution of 1.2 g. of trimethyl aldehydochitose syrup in 2.5 ml. of 8% hydrochloric acid was heated on a water bath at 80°C. for 1 hour. After cooling it was extracted 3 times with 3 ml. volumes of chloroform and the combined extract neutralised with anhydrous sodium carbonate and dried over anhydrous sodium sulphate. The mixture was filtered and the clear solution evaporated *in vacuo* at 40°C. to a syrup. The product was dissolved in an aqueous alcoholic solution of semicarbazide containing 0.66 g. of semicarbazide hydrochloride and 0.81 g. of hydrated sodium acetate which was allowed to evaporate spontaneously in air at room temperature. Clusters of long needles were thus obtained and these were recrystallised from methyl alcohol.

Melting point of the purified product ... 148°C.

Mixed melting point with trimethyl aldehydochitose semicarbazone ... ... 148°C.

No other derivative was isolated on working up the mother liquors.

Preparation of trimethyl aldehydochitose by the usual method employed in the preparation of methylated aldehydohexoses. (Levene and Meyer, J. Biol. Chem., 69, 175).

Chitose diethylmercaptal.

20 g. of dry amorphous chitose were dissolved in 22 ml. of cold fuming hydrochloric acid and 22 ml. of ethyl mercaptan added. The mixture was cooled in an ice bath to 0°C. and the shaken vigorously, recooling to 0°C. every few minutes. After approximately half an hour of this treatment the reaction mixture was left in the ice bath for 3 hours. The solution was then neutralised by addition of an excess of lead carbonate and the mass dried by shaking it with 50 g. of anhydrous sodium sulphate and leaving overnight. After twice extracting the mass with 100 ml. of dry acetone the combined extract was evaporated at 35°C. in vacuo to a stiff syrup. Weight of the product was 21.5 g. It did not crystallise. The substance was purified to a certain extent by taking up in 200 ml. of dry ethyl acetate, filtering off the small quantity which did not dissolve and adding to the cold filtrate a small quantity of charcoal. The mixture was

allowed to stand overnight at room temperature in contact with anhydrous sodium sulphate, filtered and the clear solution evaporated *in vacuo* at 40°C. to a reddish yellow syrup. A specimen of the syrup was dried to constant weight in high vacuum at 50°C.

Analysis ---	C.	H.	S.
found ...	44.83%	7.43%	22.95%
Chitose diethyl-mercaptal requires...	44.78%	7.46%	23.88%

Rotation -- $(\alpha)_D^{15^\circ}$ = +60.38° in acetone. c = 2.153%.

Methylation of chitose diethylmercaptal by Haworth's method.

A solution of 20 g. of chitose diethylmercaptal syrup in 20 ml. of acetone was methylated with 71.4 ml. of dimethyl sulphate and 215 ml. of 30% caustic soda solution, the water bath temperature being maintained at 70°C. throughout the process. Vigorous stirring was maintained during the reaction. First was added 40 ml. of the alkali solution and then dropwise at an equivalent rate the dimethyl sulphate and the remainder of the alkali were simultaneously added, the process being completed in half an hour. The mixture was immediately cooled by the addition of ice and extracted with three 70 ml. volumes of chloroform. The combined extract was freed from any excess of dimethyl sulphate by washing in a separating funnel with dilute ammonia and the chloroform layer evaporated *in vacuo* at 40°C. to a dark mobile syrup.

Distillation in high vacuum gave a yellow liquid, the bulk of which came over at 150-160°C./0.12 mm. Weight of this product was 13.5 g.

A second methylation of the syrup by Haworth's method was carried out exactly as described above the product (10.5 g.) distilled at 145-153°C./0.12 mm.

Methoxyl content	24.3%
Trimethyl chitose dimethyl-mercaptal requires	30.0%.

A further methylation by the method of Freudenberg was carried out as follows. 10 g. of the partially methylated syrup were dissolved in 70 ml. of dry ether and the solution left overnight in contact with freshly pressed sodium wire. After decanting off the solution it was filtered and evaporat in vacuo at 25°C. to a red syrup. The last traces of ether were removed under high vacuum at 45°C. To the reaction flask which was cooled in an ice bath, 7 ml. of methyl iodide were added and the solution set aside and allowed to attain room temperature. In half an hour the solution had become gelled due, probably, to the separation of finely divided sodium iodide. To the mixture was then added 50 ml. of dry ether and the solution was filtered through a layer of charco The resulting clear yellow liquid was evaporated in vacuo at 30°C. to a light red coloured mobile syrup which, on distillation at 0.12 mm. gave 7.5 g. of a yellow mobile syrup boiling at 135-145° C.

Methoxyl content ... ... 25.9%.

After the Freudenberg methylation had been twice repeate on the product 5 g. of a light yellow mobile syrup boiling at 135°C./0.12 mm. was obtained.

Analysis --	C.	H.	S.	OCH_3.
found ..	51.20%	8.39%	20.71%	29.7%
Trimethylchitose diethylmercaptal $C_{13}H_{26}O_4S_2$ requires	50.32%	8.39%	20.65%.	30.0%.

Rotation -- $(\alpha)_D^{20} = +32.16^\circ$ in acetone. c = 4.540%.

Preparation of trimethyl aldehydochitose semicarbazone from trimethyl chitose diethylmercaptal.

To a vigorously stirred mixture of 35 ml. of acetone, 15 ml. of water, 8 g. of trimethyl chitose diethylmercaptal and 20 g. of cadmium carbonate was added, drop by drop over a period of 1 hour, a solution of 16 g. of mercuric chloride in 40 ml. of acetone. Stirring was continued for 4 hours at room temperature with occasional additions of small quantities of fresh cadmium carbonate. The mixture was heated in a water bath for 10 minutes at 50°C., at 60°C. for a further 10 minute period and then filtered through a fresh layer of cadmium carbonate. The clear liquid was evaporated in vacuo at 35-40°C. in the presence of a little cadmium carbonate to a dry syrupy residue. By extracting the mass with 50 ml. of chloroform and evaporating down the filtered extract at

35-40°C. *in vacuo* was obtained 4.5 g. of a moderately mobile yellow syrup. To this product was added a solution of 1.7 g. (1 mol.) of semicarbazide prepared as previously described and the solution was set aside to evaporate at room temperature. The crop of long white crystalline needles which separated was recrystallised from methyl alcohol.

Weight of purified compound	1.5 g.
Melting point	148°C.
Mixed melting point with trimethyl-aldehydochitose semicarbazone...	148°C.

Oxidation of fully methylated chitose with nitric acid.

A 200 ml. conical flask containing 4.0 g. of the fully methylated chitose syrup and 30 ml. of concentrated nitric acid (d. 1.42) was immersed in a water bath and the temperature gradually raised. The initial vigorous reaction which set in at 40-45°C. was moderated from time to time by cooling the reaction flask under cold running water and when it had subsided the bath temperature was gradually raised over a period of 1 hour to 90°C. After 15 minutes at this temperature the reaction had practically ceased. Aft allowing the solution to cool it was diluted to about three times its volume with distilled water and evaporated *in vacuo* at 50°C. to a syrup. To this was added 5 ml. of water and the solution was again taken down *in vacuo* to a syrup at 50oC. The process of adding water and evaporating was

repeated 8 times to remove nitric acid almost completely. To the syrup was then added 5 ml. of dry methyl alcohol and the solvent taken off *in vacuo* at 50°C. to remove most of the water present. The resulting syrup was dissolved in 50 m of dry methyl alcohol containing 5% of dry hydrochloric acid gas and the solution refluxed on a waterbath for 4 hours. The solvent was removed *in vacuo* at 40°C. to give a mobile syrup which was again dissolved in 50 ml. of dry methyl alcohol containing 5% of hydrochloric acid and refluxed for 2 hours. This was again taken down to a syrup *in vacuo*, 20 m of pure methyl alcohol added and the solution neutralised with a little silver oxide. The mixture was filtered and the clear filtrate evaporated *in vacuo* at 40°C. to a mobile syrup which was distilled in high vacuum. The only product coming over at a fixed temperature was 2 g. of a colourless mobile syrup boiling at 100-103°C./0.10 mm. The product did not crystallise.

Analysis --	C	H	OCH_3
found...	50.00%	7.45%	51.1%.

Equivalent weight .... 220.

Rotation -- $(\alpha)_D^{20^\circ} = +52.09^\circ$ in methyl alcohol.
c = 5.471%.

The syrup was quite soluble in water and organic solvents It did not reduce Fehling's solution and reacted towards N/10 caustic soda as the ester of an organic acid.

Formula of the compound calculated from above data ... ... $C_{10}H_{18}O_6$

It was considered likely to be the methyl ester of trimethyl chitonic acid.

Attempted preparation of a crystalline derivative of the oxidation product from fully methylated chitose.

(1) The acid amide.

0.5 g. of the syrup were dissolved in 5 ml. of pure methyl alcohol and the solution saturated with dry ammonia at 0°C. the reaction flask being immersed in an ice bath. The solution was left at room temperature for 14 days but no crystalline amide separated. The solvent was then removed by boiling off at 40°C. in vacuo leaving a colourless semi-mobile syrup. No crystalline material had separated from this product after a prolonged period in the refrigerator and attempts to crystallise it from a number of solvents failed.

---

(2) The free acid.

0.5 g. of the methyl ester were dissolved in 20 ml. of N/10 sodium hydroxide to give a solution which was alkaline to phenolphthalein. After leaving the solution in an incubator at 37°C. for 2 days it was no longer alkaline to phenolphthalein indicating that all the alkali had been neutralised. A further 5 ml. (excess) of N/10 alkali were then added and the solution left for a further period of 3 days at 37°C. 25 ml. of N/10 hydrochloric acid (equivalent to the alkali added) were then added and the solution evaporat in vacuo at 40°C. to a syrupy mass. This was extracted with 10 ml. of dry ether and the solution after filtering from the

crystalline residue of sodium chloride was evaporated *in vacu* at 30°C. to a colourless semi-mobile syrup. The product, however, did not crystallise.

---

(3) The calcium salt.

0.25 g. of the free acid were dissolved in 5 ml. of water and the solution was shaken with 1 g. of powdered calcium carbonate. When the evolution of carbon dioxide had practically ceased the mixture was heated on a boiling water-bath for 10 minutes to ensure complete neutralisation. On filtering and allowing the colourless filtrate to evaporate spontaneously in air at room temperature a hard glassy amorphous solid was obtained which was soluble in water and absolute alcohol but insoluble in other. It was not isolated in crystalline form.

Preparation of trimethyl chitonic acid.

Ethyl ester of chitonic acid.

5 g. of calcium chitonate, prepared from chitose by the method of Fischer (Ber. 27, 138), and purified by recrystalli ing from water, were dissolved in 50 ml. of water and to the cold vigorously stirred solution was added drop by drop 1.465 g. (1 mol.) of hydrated oxalic acid in 25 ml. of water. The resulting mixture was heated on a boiling waterbath for

10 minutes to coagulate the precipitated calcium oxalate and after filtering, the clear colourless solution was evaporated *in vacuo* at 40°C. to a dry syrup. This was taken up in 50 ml. of absolute alcohol containing 1% of dry hydrochloric acid and after leaving the solution at room temperatu overnight it was evaporated *in vacuo* at 40°C. The crystallin material in the resulting mass was extracted with ethyl acet and the residual syrup subjected to a second esterification. The ethyl acetate solution of the crystalline product from th two esterifications was then evaporated to dryness *in vacuo* at 40°C. and the crystalline product recrystallised from isopropyl alcohol.

Yield of the purified compound ... ... 4.5 g.

Melting point ... ... 106°C.

Rotation .. $(\alpha)_D^{15°}$ = +46.69° in ethyl alcohol.
c = 2.356%.

Analysis —	C	H
found ..	46.58%	6.90%.
$C_8H_{14}O_6$ requires ...	46.60%	6.80%.

Equivalent weight... ... ... 204.

Methyl ester of chitonic acid.

5 g. of calcium chitonate were treated as described in the method for preparation of the ethyl ester with the exception that absolute alcohol was replaced by dry methyl alcohol. The ethyl acetate soluble product was in this case obtained not as a crystalline derivative but as a colourless

viscous syrup.

Analysis --	C	H	OCH_3
found ..	43.65%	6.28%	15.9%
$C_7H_{12}O_6$ requires ...	43.75%	6.25%	16.15%

Trimethyl methyl chitonic ester.

3.5 g. of methyl chitonic ester were dissolved in 5 ml. of dry acetone and to the solution 5 ml. of methyl iodide were added. A further quantity of acetone, just sufficient to bring the precipitated material back into solution, was introduced and the liquid methylated with 6 g. of silver oxide. For methylation the solution was kept at a temperatur of 40°C. under a reflux condenser and the silver oxide added in 1 g. lots at intervals of half an hour. The reaction mixture was meintnined at this temperature for 5 hours and then gradually raised to 60°C. The partially methylated product was isolated in the usual manner and subjected to five more methylations, the addition of acetone being left out after the second treatment as the product at this stage was quite soluble in methyl iodide. On distillation of the final product in high vacuum most of the colourless mobile syrup came over at 100-103°C./0.1 mm. The yield was 3.3 g.

Analysis --	C	H	OCH_3
found ..	52.00%	7.68%	52.2%
$C_{10}H_{18}O_6$ requires.	51.28%	7.69%	53.0%.

Equivalent weight ... ... 230.

Rotation -- $(\alpha)_D^{20^\circ}$ = + 55.35° in methyl alcohol.
c = 5.023%.

Trimethyl chitonic acid.

2.3 g. of trimethyl methyl chitonic ester were dissolved in 100 ml. (a slight excess) of N/10 sodium hydroxide and the solution left in the incubator at 37°C. for 2 days. 100 ml. of N/10 hydrochlorid acid were then added and the solution evaporated <u>in vacuo</u> at 30°C. to a light yellow moderately mobile syrup which did not crystallise. Weight of the syrup was 2.13 g. It distilled in high vacuum at 124-127°C./0.1 mm as a colourless moderately mobile syrup.

Analysis -- OCH_3.
found ... 42.5%.
$C_9H_{16}O_6$ requires ... 42.3%.
Rotation -- $(\alpha)_D^{20^\circ}$ = +69.89° in water.
c = 5.466%.

<u>Attempted preparation of a crystalline derivative of trimethyl chitonic acid.</u>

Calcium salt.

2.5 g. of trimethyl chitonic acid were dissolved in 25 ml. of distilled water and to this was added 2.5 g. of powdered calcium carbonate. The mixture was left at room

temperature until evolution of carbon dioxide had ceased. It was then brought to boiling point, filtered and the clear solution left to evaporate spontaneously at room temperature. Crystallisation did not take place and only a hard glassy amorphous mass was obtained.

Sodium salt.

To 2.39 g. of the calcium salt in 25 ml. of water was added dropwise and with stirring 0.63 g. (1 mol.) of hydrated oxalic acid in 10 ml. of water. The mixture was heated to boiling point to coagulate the precipitated calcium oxalate, filtered and the clear solution of trimethyl chitonic acid was just neutralised with N/1 caustic soda solution using a drop of phenolphthalein as indicator. The liquid was left to evaporate spontaneously in air but again only a clear brittle amorphous mass was obtained.

The acid amide.

1.1 g. of trimethyl (methyl) chitonic ester were dissolv in 10 ml. of dry methyl alcohol and the solution saturated in an ice bath with dry ammonia. No crystallisation occurred when this solution was left at room temperature for 14 days. Subsequent removal of the solvent under diminished pressure at 35°C gave a viscous syrup which did not crystallise.

Phenylhydrazide.

To 1 g. of the free acid dissolved in 25 ml. of water was added 0.49 g. (1 mol.) of phenylhydrazine. No crystalline material separated from the resulting clear solution and on evaporation at 40°C. under diminished pressure a red oily uncrystallisable residue was obtained.

Behaviour of fully methylated chitose in methyl alcohol.

5.677 g. of fully methylated chitose syrup of boiling point 100°C./0.1 mm. was weighed into a 100 ml. graduated flask dissolved in and made up to the graduation mark with dry methyl alcohol. There was a gradual fall in rotatory power over a period of three days when the solution was left at room temperature as indicated in the following table.

Time after dissolution in methyl alcohol.	Rotation of the solution in a 1 dm. tube.
0	-
5 mts.	+1.09°
30 "	+1.06°
2 hours	+1.04°
7 "	+0.93°
24 "	+0.87°
48 "	+0.84°
72 "	+0.83°
96 "	+0.83°
240 "	+0.83°

After 10 days when the solution had apparently attained a constant rotation the solvent was removed at 40°C. in vacu

and the resulting mobile syrup distilled in high vacuum. 5.2 g. of a colourless mobile liquid boiling at 100°C./0.1 mm. were thus obtained.

Methoxyl content of the product	57.4%.
" " " " original fully methylated chitose...	56.7%.

Attempted preparation of trimethyl chitose dimethyl acetal from trimethyl aldehydochitose.

5 g. of fully methylated chitose prepared as previously described, were dissolved in 100 ml. of N/10 sulphuric acid and the solution heated on a boiling waterbath for 1 hour. The solution was cooled, neutralised by shaking with an excess of barium carbonate, and filtered. The colourless filtrate was twice extracted with 30 ml. volumes of chloroform and the combined extract after drying with anhydrous sodium sulphate was evaporated *in vacuo* at 40°C. to a moderately mobile syrup. On distilling the material in high vacuum 2.1 g., the main fraction, came over at 120°C./0.1 mm. as a moderately mobile syrup. 0.716 g. of this fraction shown in a previous experiment to consist mainly of trimethyl aldehydochitose were dissolved in 15 ml. of dry methyl alcohol. The rotation of +2.13° in a 1 dm. tube observed immediately after making up the solution remained unaltered over a period of 24 hours at room temperature, 1 ml. of 15% hydrochloric acid in dry

methyl alcohol was then added whereupon a fall in rotation at room temperature was observed as indicated in the followi table.

Time after addition of hydrochloric acid to the solution.	Observed rotation i a 1 dm. tube.
5 mts.	+1.59°
1 hour	+1.55°
6 hours	+1.47°
12 "	+1.42°
24 "	+1.35°
36 "	+1.32°
48 "	+1.30°
60 "	+1.27°
100 "	+1.27°

After 100 hours when the rotation had reached a steady value the solution was neutralised by shaking with silver carbonate, filtered and evaporated to a mobile syrup in vacuo at 40°C. Distillation in high vacuum yielded 0.5 g. of a colourless mobile syrup boiling at 100°C./0.1 mm.

Methoxyl content of the product	57.1%.
" " " " original fully methylated chitose	56.7%.
Methoxyl content of trimethyl-chitose dimethylacetal.	62.0%.

EXAMINATION OF "METHYL CHITOSIDE" SYRUP.

A fraction of the syrup, unlike free chitose, was found to be quite soluble in both ethyl acetate and acetone. 20 g. of the crude syrup was repeatedly extracted with hot ethyl acetate, the combined extract heated at 40°C. with a little charcoal and the cooled mixture left for 24 hou in contact with anhydrous sodium sulphate. After filtering, the clear solution was evaporated *in vacuo* at 40°C. to a viscous red syrup which was left at that temperature and at 0.1 mm. pressure until no further loss in weight was detected. The weight of ethyl acetate soluble fraction obtained was 10.1 g.

Part of this fraction was found to be quite soluble in ether, the remainder being almost completely insoluble. On shaking the ethyl acetate soluble fraction with an equal volume of dry ether a clear mobile homogeneous solution was formed but on addition of a further quantity of ether the liquid separated into two phases; a light greenish yellow ethereal solution and a viscous insoluble syrup. The addition of small quantities of ether was continued with vigorous shaking of the mixture until no further precipitate was obtained from the ethereal solution. The latter was the decanted off and left for 24 hours in contact with anhydrous sodium sulphate. The solution was filtered and evaporated in vacuo at 25°C. to a viscous syrup which was dried in high vacuum to constant weight at 30°C. The weight of the

ether soluble product was 3.2 g.

The ether insoluble residue was then taken up in 200 ml. of dry ethyl acetate and the solution left overnight in contact with anhydrous sodium sulphate and a little charcoal. After filtering the clear liquid was evaporated in vacuo at 40°C. to a viscous light red syrup which was maintained at that temperature at a pressure of 0.1 mm. until no further loss in weight was detected. The weight of the product was 6.1 g.

20 g. of the crude syrup were thus separated into the three distinct fractions --

F_1 ... Insoluble in ethyl acetate.

F_2 ... Soluble in ethyl acetate, insoluble in ether.

F_3 ... Soluble in ethyl acetate, soluble in ether.

Examination of F_1.

5 g. of the ethyl acetate insoluble fraction were dissolved in 100 ml. of N/1 sulphuric acid to give a dark coloured solution which could not be observed polarimetrically One half of the solution was heated on a boiling waterbath for 1 hour and the other half left for 7 days at room temperature. Each solution was then neutralised by shaking with barium carbonate, filtered and evaporated in vacuo at 40°C. to a fairly mobile syrup which was then taken up in 70 ml. of 96% alcohol. To each was added 1.8 g. of

2;4dinitrophenylhydrazine and the solution treated exactly as previously described in the preparation of chitose 2;4dinitrophenylhydrazone. In neither case did any hydrazone crystallise from the dark red oily product obtained, even afte seeding with a few crystals of chitose 2;4dinitrophenylhydrazo

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Examination of F_3.

2.5 g. of the ether soluble fraction were dissolved in 50 ml. of water to give a somewhat turbid solution which was cleared by filtration. The rotation of the resulting light yellow coloured liquid in a 1 dm. tube was - 0.86° immediately after filtration and remained unaltered over a period of 48 hours at room temperature. When 0.5 g. of concentrated sulphuric acid in 1 ml. of water was then added, giving the solution a content of approximately 1% sulphuric acid, the gradual fall in laevorotatory power indicated in the following table, was observed over a period of 10 days.

Time after acidifying the solution.	Observed rotation in a 1 dm. tube.
15 mtes.	-0.85
6 hours	-0.82
12 "	-0.78
24 "	-0.72
48 "	-0.59
72 "	-0.49
96 "	-0.44
120 "	-0.40

Time after acidifying the solution.	Observed rotation in a1 dm. tube.
144 hours	0.36
168 "	0.34
192 "	0.33
216 "	0.33
240 "	0.33

After 10 days when the rotation of the solution had attained a constant value it was neutralised by shaking with barium carbonate and filtered. The solution, unlike the original neutral aqueous solution of F_3 gave a positive Fehling's reaction. It did not restore the colour to Schiff's reagent.

An attempt to prepare the crystalline chitose 2:4dinitrophenylhydrazone from the syrupy product obtained by evaporation of the hydrolysed solution in vacuo at 40°C. was however unsuccessful, only a red uncrystallisable oil being obtained.

Examination of F_2.

An aqueous solution of the syrup did not reduce Fegling's solution but after hydrolysis by heating with N/10 hydrochloric acid it gave astrong Fehling's reaction.

4 g. of the dry syrup were dissolved in water and the solution made up to 50 ml. A slight turbidity present in the liquid was removed by filtration. The rotation (+1.65°)

of the neutral solution in a 1 dm. tube remained constant over a period of 10 days. 0.5 g. of concentrated sulphuric acid in 1 ml. of water was then added to give an approximate concentration of 1% sulphuric acid and the following changes in rotation were observed --

Time after acidifying the solution.	Rotation in a 1 dm. tube.
20 mts.	+1.61°
1 hour 40 "	+1.54°
2 " 25 "	+1.49°
4 " 0 "	+1.46°
8 " 30 "	+1.45°
17 " 45 "	+1.45°
27 " 0 "	+1.48°
42 "	+1.53°
49 "	+1.54°
65 "	+1.57°
99 "	+1.60°
123 "	+1.62°
144 "	+1.64°
168 "	+1.67°
193 "	+1.70°
216 "	+1.72°
241 "	+1.74°
291 "	+1.76°
338 "	+1.77°
410 "	+1.77°
480 "	+1.77°

After 20 days at room temperature the solution was neutralised with barium carbonate, filtered and evaporated in vacuo at 40°C. to a mobile syrup. Following the directions

previously described for the preparation of chitose 2:4dinitrophenylhydrazone 1.2 g. of a yellow crystalline product was prepared from this syrup. Purified by re-crystallising from absolute alcohol it had a melting point of 175°C. No depression of melting point was observed after it was intimately mixed with a specimen of pure chitose 2:4dinitrophenylhydrazone.

	C	H	OCH_3
Analysis of F_2 ...	47.64%	7.66%	24.3%
Methyl chitoside requires	47.73%	6.82%	17.6%
Chitose dimethylacetal requires	46.15%	7.69%	29.8%.

Rotation ... $(\alpha)_D^{20^\circ}$ = +20.3° in water. c = 5.130%.

The effect of treatment in dry methyl alcoholic hydrochloric acid on the methoxyl content of F_2.

1.4 g. of the dry ethyl acetate soluble, ether insoluble fraction of "methyl chitoside" syrup were dissolved in 30 ml. of a 1% hydrochloric acid solution in dry methyl alcohol and the clear reddish yellow liquid left at room temperature for 24 hours. The initial rotation of the solution (+0.66°) remained practically unaltered during this period. The solution was neutralised by shaking with dry silver carbonate, the mixture filtered through a layer of charcoal and the light yellow coloured filtrate evaporated in vacuo at 40°C. to a viscous yellow syrup. The product was

dried to constant weight in high vacuum.

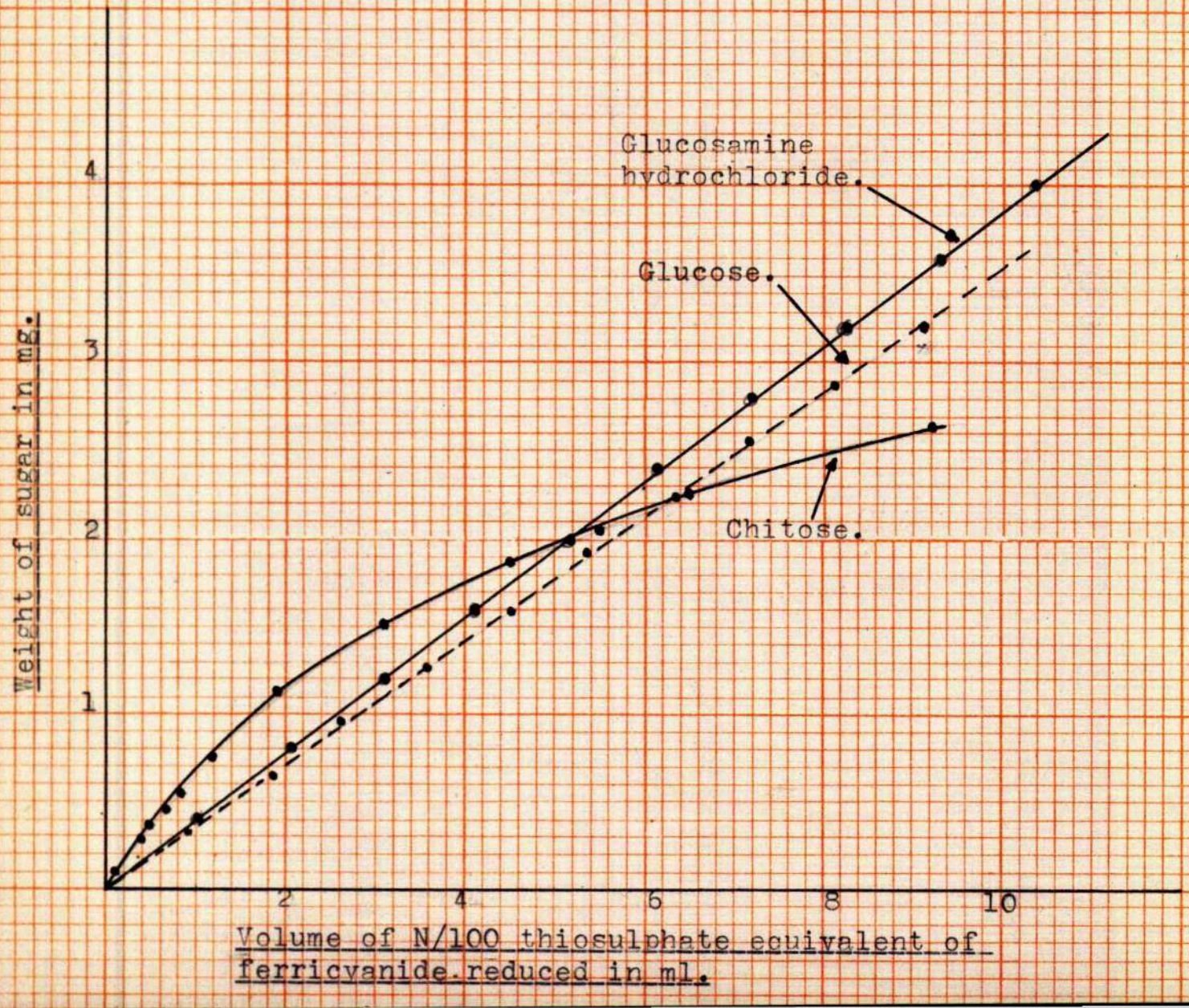
Methoxyl content ... ... 24.8%.
Methoxyl content of F_2... 24.3%.

Preparation of trimethyl aldehydochitose semicarbazone from F2.

2 g. of the dry ethylacetate soluble ether insoluble fraction were methylated by the method of Purdie following the directions previously described for the methylation of the crude "methyl chitoside" and from the product by distillation in high vacuum was obtained 1.4 g. of an almost colourless mobile syrup distilling at 100°C./0.1 mm. That this product was fully methylated chitose was demonstrated by the fact that it had a methoxyl content of 57.0% and that 0.8 g. of trimethyl aldehydochitose semicarbazone was obtained by treatment of the hydrolysed solution of 1.3 g. of the syrup with semicarbazide.

GRAPH 3.

The oxidation of glucose, glucosamine hydrochloride and chitose by Hanes' A solution.



ESTIMATION OF CHITOSE.

Attempted estimation of chitose in aqueous solution by Hanes' method.

Standard aqueous solutions of glucose (0.32 mg. per ml. and glucosamine hydrochloride (0.40 mg. per ml.) were prepared and the results of a series of estimations by Hanes' method carried out on varying measured volumes of these solutions are indicated in the following tables and graph 3.

Volume of Glucose solution in ml.	Weight of Glucose in mg.	Vol. of N/100 thiosulphate equivalent ferricyanide reduced in ml.
1	0.32	0.90
2	0.64	1.85
3	0.96	2.60
4	1.28	3.55
5	1.60	4.50
6	1.92	5.35
7	2.24	6.35
8	2.56	7.15
9	2.88	8.10
10	3.20	9.10

Volume of glucosamine hydrochloride solution in ml.	Wt. of glucosamine hydrochloride in mg.	Vol. of N/100 thiosulphate equivalent of ferricyanide reduced in ml.
1	0.40	1.00
2	0.80	2.05
3	1.20	3.10
4	1.60	4.10
5	2.00	5.15
6	2.40	6.15
7	2.80	7.20
8	3.20	8.25
9	3.60	9.30
10	4.00	10.35

It is apparent from these results that for both glucose and glucosamine hydrochloride the quantity of ferricyanide reduced is directly proportional to the amount of sugar present.

A solution of 2.500 g. of glucosamine hydrochloride in water was made up to 20 ml. and deaminated at room temperature by the addition of 1.0 g. (1½ mols.) of sodium nitrite. After 4 days when nitrogen evolution was no longer apparent the solution was heated to 50°C. on a waterbath for 1½ hours to complete the deamination as far as possible. 3 ml. of N/1 hydrochloric acid solution (slightly more than the equivalent of the excess sodium nitrite used in the deamination) were added to the cold solution which was then aerated vigorously for 30 minutes to remove nitrous

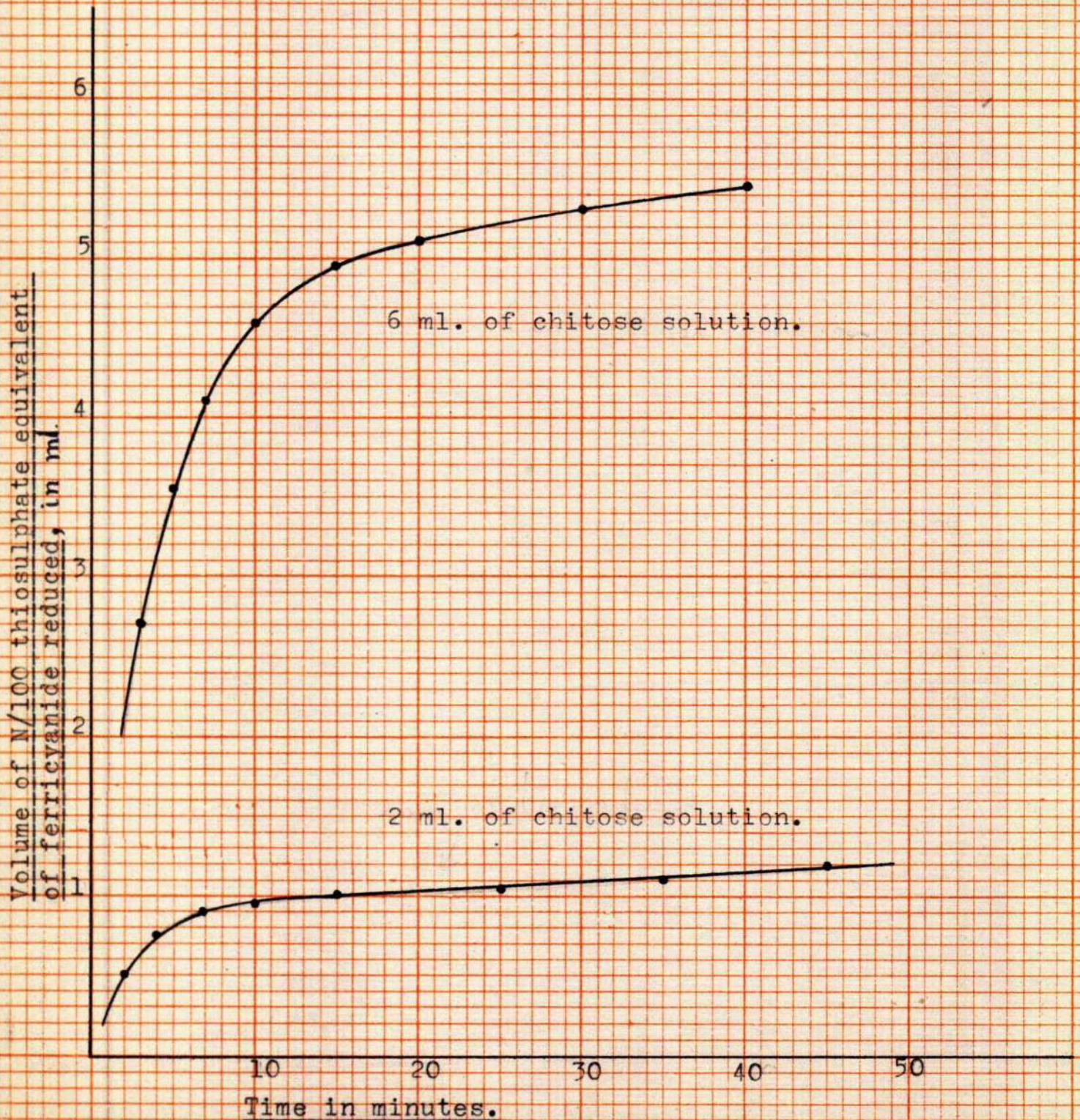
acid. This solution containing theoretically 1.8795 g. of chitose was made up to 25 ml. in a graduated flask. From this a standard solution of chitose was made up by diluting 5 ml. to 1 litre in a graduated flask. This standard solution contained theoretically 0.375 mg. of chitose per ml.

To test the applicability of Hanes' method for the estimation of chitose the ordinary procedure was adopted using measured volumes of the standard solution prepared as described in the preceding paragraph. The results are shown in the following table and graph 3.

Volume of chitose solution in ml.	Theoretical wt. of chitose in mg.	N/100 thiosulphate equivalent of ferricyanide reduced in ml.
0.25	0.094	0.15
0.50	0.188	0.35
0.75	0.281	0.40
1.00	0.375	0.50
1.25	0.469	0.65
1.50	0.562	0.85
2.00	0.750	1.15
3.00	1.125	1.90
4.00	1.500	3.10
5.00	1.875	4.50
5.50	2.063	5.50
6.00	2.250	6.50
7.00	2.625	9.20

GRAPH 4.

The rate of oxidation of chitose by Hanes' A solution.



Rate of reduction of the ferricyanide of Hanes' A solution by chitose.

The reaction was carried out by heating the chitose solution with Hanes' A solution in a waterbath at 70°C. for varying intervals of time and the results of a series of experiments are indicated in the following tables and Graph 4.

Reduction of Hanes' A solution by 2 ml. of chitose solution:-

Reaction time in minutes.	N/100 thiosulphate equivalent of ferricyanide reduced, in ml.
2	0.50
4	0.75
7	0.90
10	0.95
15	1.00
25	1.05
35	1.10
45	1.20

Reduction of Hanes' A solution by 6 ml. of chitose solution:-

Reaction time in minutes.	N/100 thiosulphate equivalent of ferricyanide reduced, in ml.
3	3.70
5	3.55
7	4.10
10	4.60
15	4.95
20	5.10
30	5.30
40	5.45

Oxidation of chitose by ferricyanide solutions of varying pH value.

4.1250 g. of potassium ferricyanide and 32.40 g. of hydrated sodium acetate were dissolved in water and the solution made up to 500 ml. in a graduated flask. To each of 5 ml. volumes of this solution were added 10 ml. of water, 5 drops of phenol red indicator and varying amounts of a solution of 0.5% acetic acid. The pH value of each solution was estimated by comparison of the colour in a comparator box with standard buffer solutions containing the same concentration of phenol red indicator. The variation of pH with the amount of acetic acid present was as follows:

Vol. of acetic acid added.	pH of the solution.	Vol. of acetic acid added.	pH of the solution.
0 ml.	7.5	0.25 ml.	6.6
0.10 "	6.9	0.30 "	6.5
0.15 "	6.8	0.60 "	6.4
0.20 "	6.7	0.80 "	6.3

To each of a number of 5ml. volumes of the standard chitose solution was added 5 ml. of water, 5 ml. of the above potassium ferricyanide sodium acetate solution and a measured volume of 0.5% acetic acid solution. Each solution was heated for 15 minutes in a boiling water-bath, cooled rapidly in cold running water and the extent of ferricyanide reduction estimated by the ordinary Hanes'

method. The results are indicated in the following table.

Vol. of 0.5% acetic acid added in ml.	Estimated pH of the solution.	N/100 thiosulphate equivalent of ferricyanide reduced, in ml.
0	7.5	1.05
0.1	6.9	0.90
0.2	6.7	0.80
0.6	6.4	0.66

Rate of reduction of the above ferricyanide-sodium acetate solution at 100°C. by chitose solution.

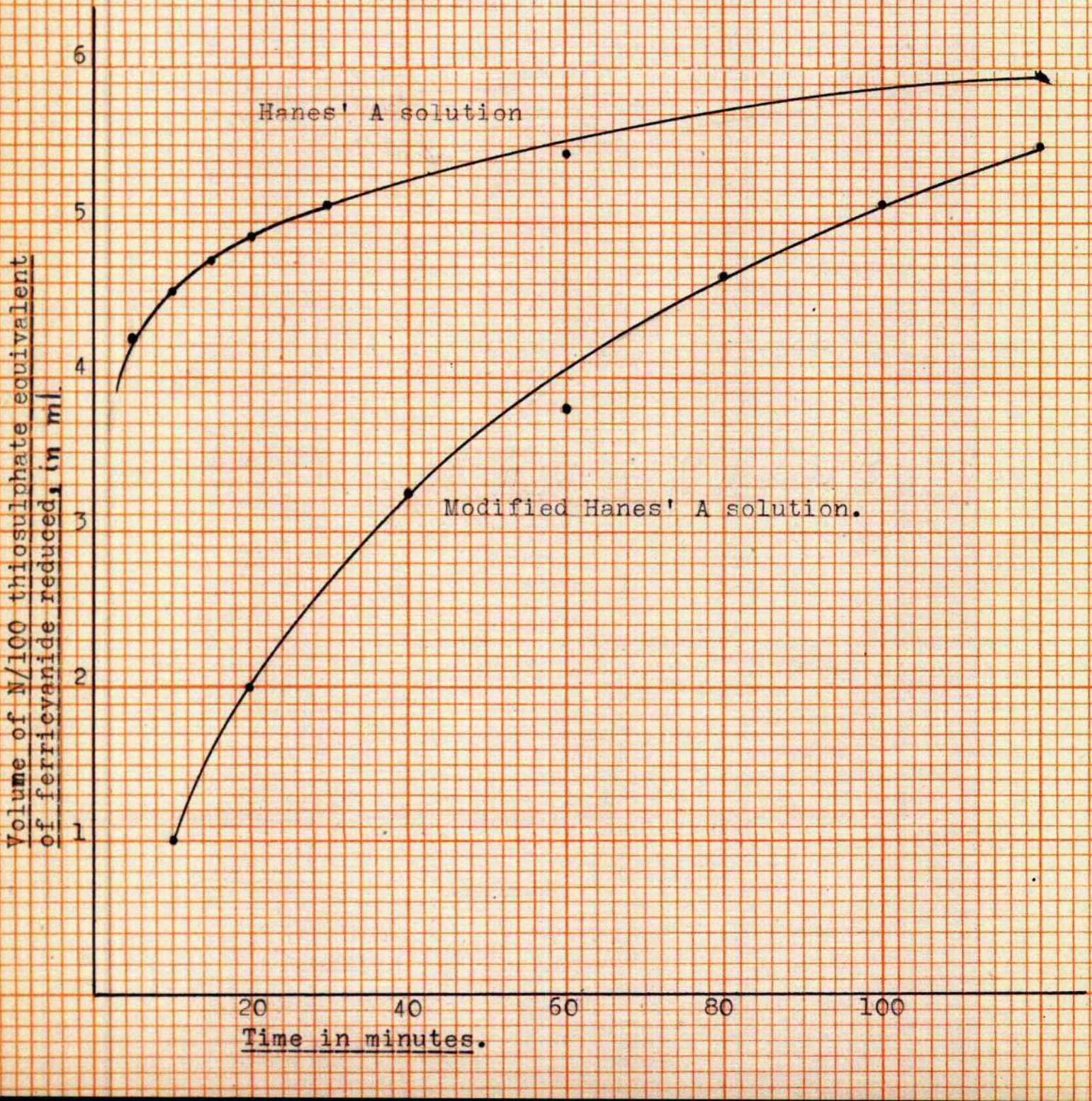
Into each of a series of test tubes was pipetted 5 ml. of the standard chitose solution, 5 ml. of distilled water and 5 ml. of the potassium ferricyanide-sodium acetate solution. The tubes were immersed in a boiling waterbath and at varying intervals of time transferred to a bath of running cold water and the extent of ferricyanide reduction estimated by Hanes' method.

To compare the rate of oxidation with that obtained with Hanes' A solution the experiment was repeated, the 5 ml. of ferricyanide-sodium acetate solution being replaced by 5 ml. of Hanes' A solution.

The following tables and graph 5 indicate that the ferricyanide-sodium acetate solution is reduced at a much slower rate than in the case where Hanes' A solution is employed.

GRAPH 5.

The effect of lowering the pH value on the oxidation of chitose by Hanes' A solution.



Rate of oxidation of chitose by ferricyanide-sodium acetate solution —

Reaction time in minutes.	N/100 thiosulphate equivalent of ferricyanide reduced in ml.
10	1.00
20	2.00
40	3.25
60	3.80
80	4.65
100	5.10
120	5.50
180	6.30

Rate of oxidation of chitose by ferricyanide-sodium carbonate (Hanes' A) solution —

Reaction time in minutes.	N/100 thiosulphate equivalent of ferricyanide reduced in ml.
5	4.25
10	4.55
15	4.75
20	4.90
30	5.10
60	5.45
120	5.93

Effect of the presence of small quantities of sodium nitrite or nitrous acid on oxidation of chitose by Hanes' A solution

5 ml. of the standard chitose solution were pipetted into each of 3 reaction tubes. To tube I was added 5 ml. of water. To tube II was added 5 ml. of sodium nitrite solution (0.2 mg. of sodium nitrite per ml.). To tube III was added 4 ml. of the same sodium nitrite solution and 1 ml. of N/100 hydrochloric acid to produce free nitrous acid in solution. To each was then added 5 ml. of Hanes' A solution. The tubes were heated in a boiling waterbath for 15 minutes, cooled in cold running water and the extent of ferricyanide reduction estimated in each case by Hanes' method. In every case the extent of ferricyanide reduction was found to be equivalent to 4.95 ml. of N/100 thiosulphate solution indicating that the presence of small amounts of nitrite such as may be present in chitose solutions does not interfere with the amount of ferricyanide reduced in this estimation method.

Preparation of chitose 2:4dinitrophenylhydrazone directly from an aqueous solution of chitose.

A solution of 5 g. of glucosamine hydrochloride in water deaminated as described in the previous series of experiments in which Hanes' method was found to be inapplicable for the estimation of chitose and made up to

50 ml. contained theoretically 3.759 g. of chitose. 19.3 ml. of this solution containing theoretically 1 g. of chitose was shaken vigorously at room temperature with 1.5 g. (excess) of 2:4dinitrophenylhydrazine dissolved in 100 ml. of nitrobenzene. Light yellow crystals of chitose 2:4dinitrophenylhydrazone gradually separated from the mixture which, after 24 hours, was removed from the shaker, cooled overnight in the refrigerator and filtered through a Gooch crucible with suction. The crystalline product was washed with a little ether and dried in a vacuum desiccator over concentrated sulphuric acid.

Weight of the dry product	0.901 g.
Melting point	170-171°C.
Mixed melting point with pure chitose 2:4dinitrophenylhydrazone	170-172°C.

The mixed solution of nitrobenzene and water obtained as the filtrate were again shaken together vigorously for a period of 12 hours but no further yield of the crystalline hydrazone was obtained. It was concluded that practically the whole of the chitose had been converted to chitose 2:4dinitrophenylhydrazone in the first shaking. Since chitose 2:4dinitrophenylhydrazone is very insoluble in cold water and almost completely insoluble in cold nitrobenzene the chitose content of a solution could be estimated approximately by this method.

Theoretical yield of hydrazone from 1 g. of chitose	2.111 g.

Weight of the hydrazone obtained ... 0.901 g.
Chitose content of the solution ... 42.7%.

Attempted estimation of the chitose content of a solution by shaking with solid 2:4dinitrophenylhydrazine and finding the increase in weight of solid material.

To 6.65 ml. of the chitose solution prepared in the previous experiment was added a weighed excess (0.5991 g.) of dry finely powdered 2:4dinitrophenylhydrazine and the mixture shaken at room temperature for 48 hours. The resulting solid material contained a considerable amount of a light yellow crystalline substance apart from the darker red grains of the original 2:4dinitrophenylhydrazine. The mixture was filtered through a Gooch crucible containing a closely fitting piece of filter paper and dried to constant weight in a hot air oven at 100°C.

Tare of Gooch	5.0567 g.
Wt. of Gooch + dry solid.	5.8459 g.
Wt. of solid material...	0.7892 g.
Wt. of 2:4dinitrophenylhydrazine used....	0.5991 g.
∴ increase in weight was	0.1901 g.

This increase in weight can be considered due to the addition of the chitose unit in the formation of crystalline chitose 2:4dinitrophenylhydrazone, which can

be represented by the following equation --

$C_6H_{10}O_5 + NH_2.C_6H_4N_3O_4 \longrightarrow H_2O + C_6H_{10}O_4:N.C_6H_4N_3O_4$

It was calculated from the molecular weights of the materials involved that 162 g. of chitose combine with 198 g. of 2:4dinitrophenylhydrazine to give 342 g. of chitose 2:4dinitrophenylhydrazone so that in the quantitative convertion of 198 g. of 2:4dinitrophenylhydrazine to chitose 2:4dinitrophenylhydrazone there is an increase in weight of 144 g. (342 - 198 g.) equivalent to the addition of 162 g. of chitose. Therefore an increase in weight of the solid of 1 g. is equivalent to 1.125 g. of chitose.

Actual increase in wt., observed ... 0.1901 g.

∴ equivalent wt., of chitose ..... 0.1901 x 1.125 g.
= 0.2139 g.

Theoretical wt., of chitose in 6.65 ml. of the solution ... ... 0.5000 g.

∴ chitose content ... ... = 42.8%.

This value is in close agreement with that obtained in the previous experiment (42.7%).

<u>Determination of the accuracy of the chitose estimation method described in the preceding experiment.</u>

0.7880 g. of the dry solid product obtained in the preceding experiment was removed from the Gooch crucible and freed from unchanged 2:4dinitrophenylhydrazine by

shaking at room temperature in a flask with 100 ml. of nitrobenzene which had been previously saturated at room temperature with pure chitose 2:4dinitrophenylhydrazone and filtered. After 12 hours shaking the mixture was filtered through a tared Gooch crucible washed with a little ether and dried to constant weight in an oven at 60°C.

Weight of Gooch+dry product ... = 5.5082 g.
Tare of Gooch ... ... = 5.0560 g.
Weight of the dry product. ... = 0.4522 g.

0.4522 g. of chitose 2:4dinitrophenylhydrazone ... ... ≡ 0.2142 g. chitose.

0.7880 g. of the original solid ≡ 0.2142 g. of chitose.

∴ 0.7892 g. of the original solid ≡ 0.2145 g. of chitose.

Weight of chitose estimated in preceding experiment ... ... = 0.2139 g.

Melting point of the product. = 170°C.

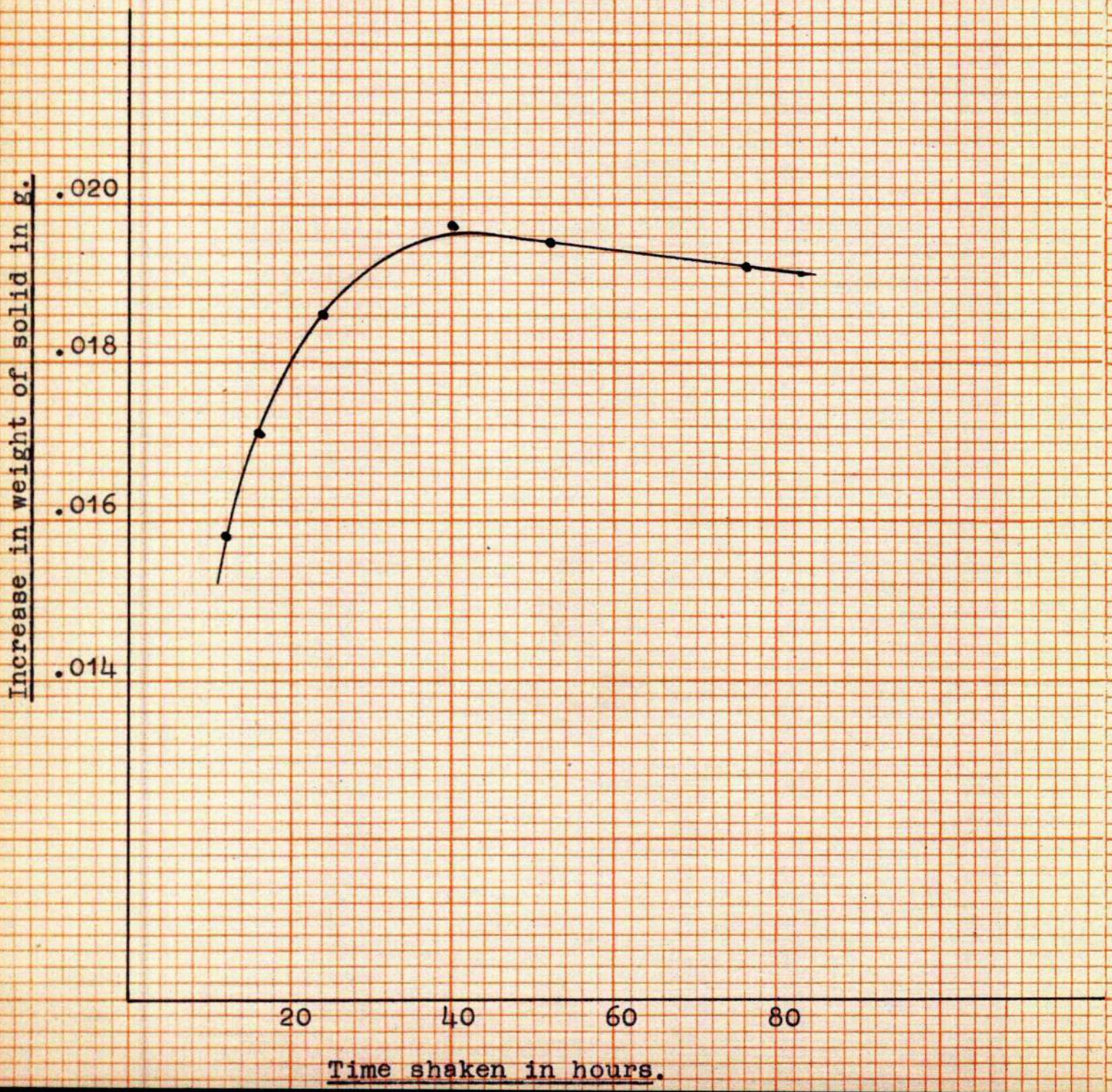
Mixed melting point with pure chitose 2:4dinitrophenylhydrazone = 171-172°C.

<u>Rate of formation of crystalline chitose 2:4dinitrophenylhydrazone in an aqueous solution of chitose shaken with solid, finely powdered 2:4dinitrophenylhydrazine.</u>

Into each of a series of small test tubes was accurately weighed about 0.05 g. of pure finely ground 2:4dinitrophenylhydrazine and into each was pipetted 0.5 ml. of a chitose solution and 0.5 ml. of water. The chitose

GRAPH 6.

The rate of hydrazone formation on shaking a solution of chitose with finely ground 2:4dinitro--phenylhydrazine.



No. of tube.	1	2	3	4	5	6
Wt. of tube.	3.9362 g.	4.0624 g	3.6532 g	3.6666 g	3.8616 g	4.0162 g
Wt. of tube + hydrazine.	3.9874 -	4.1164 -	3.7060 -	3.7114 -	3.9117 -	4.0655 -
Wt. of hydrazine.	.0512 -	.0540 -	.0528-	.0448 -	.0501 -	.0493 -
Time shaken, in hours.	12.	16	24	40	52	76
Tare of Gooch.	8.6472 g	5.9652 g	5.9665 g	8.0756 g	5.9677 g	8.0764 g
Wt. of Gooch-dry product	8.7124 -	6.0344 -	6.0358 -	8.1379 -	6.0351 -	8.1428 -
Wt. of dry product.	.0652 -	.0692 -	.0693 -	.0623 -	.0674 -	.0664 -
Increase in wt. of solid	.0140 -	.0152 -	.0165 -	.0175 -	.0173 -	.0171 -
Wt. of chitose equivalent to the wt. increase.	.0158 -	.0171 -	.0186 -	.0197 -	.0195 -	.0192 -
Chitose content of the solution expressed as a percentage of the theoretical amount (0.0376 g.	42.1%	45.6%	49.5%	52.5%	52.0%	51.5%

solution used in this experiment was prepared by deaminating a solution of 2.5 g. of glucosamine hydrochloride in 20 ml. of water with 0.8 g. (1 mol.) of sodium nitrite in the refrigerator at 0°C. for two days and then at room temperature for 4 days. The resulting chitose solution was made up to 25 ml. in a graduated flask and contained theoretically 0.0752 g. per ml. The reaction tubes were shaken at room temperature for varying lengths of time after which the contents of each was filtered through a tared Gooch crucible which was then dried to constant weight in a hot air oven at 100°C. In each case the amount of chitose converted to crystalline chitose 2:4dinitrophenylhydrazone was calculated from the increase in weight of the solid. The results of the experiment are indicated in the ~~following~~ appended table and graph 6.

Attempted preparation of other crystalline hydrazones of chitose.

Phenylhydrazone.

25 ml. of chitose solution were prepared by deamination of 2.5 g. of glucosamine hydrochloride as described in the preceding experiment. The solution was shaken with 1.2 g. of redistilled phenylhydrazine, most of which dissolved immediately and the clear liquid was left in the refrigerator for several days. No crystalline material separated from

from solution. On adding an equal volume of ethyl acetate and vigorously shaking the mixture the red colour of the aqueous solution was almost completely transferred to the ethyl acetate layer. The ethyl acetate extract was twice washed with 10 ml. of water, treated with a little charcoal, dried over anhydrous sodium sulphate, and filtered. As the clear light red coloured solution evaporated spontaneously in air at room temperature a red oil separated. Attempts at recrystallising the product from a number of solvents were unsuccessful.

p-tolylhydrazone.

To 25 ml. of an aqueous chitose solution prepared as described above was added 1.7 g. (1 mol.) of p-tolylhydrazine hydrochloride, 1 g. of sodium acetate and just sufficient glacial acetic acid to form a clear solution. After standing overnight the solution was extracted with ethyl acetate and the extract treated as in the previous experiment. Again only an uncrystallisable oily product was obtained.

p-Bromophenylhydrazone and methylphenylhydrazone.

The above procedure was repeated withp-bromophenyl-hydrazine hydrochloride and methylphenylhydrazine sulphate but in neither case did a crystalline product result.

Chitose phenylhydrazone p-sulphonic acid.

2.2 g. of phenylhydrazine p-sulphonic acid were dissolved in 25 ml. of chitose solution. This was left overnight at

room temperature. No product was removed by extraction with ethyl acetate and on allowing the aqueous solution to evapore spontaneously at room temperature only a dark tarry mass was obtained.

Crystalline benzylphenylhydrazone of chitose.

25 ml. of chitose solution were shaken vigorously with 10 ml. of benzene containing 2.2 g. of benzylphenylhydrazine. A white crystalline compound gradually separated in fine needles from the mixture. After shaking for 6 hours the solid material was removed by filtration, washed with a few ml. of benzene and allowed to dry in air.

Weight of the crude product ... ... 1.71 g.

Yield ... ... 53.3% of the theoretical amount from 2.5 g. of glucosamine hydrochloride.

Melting point of the crude hydrazone ... 77-80°C.

The substance was found to be very sparingly soluble in cold water but moderately soluble in hot solution. Purification was attempted by dissolving in water at 55-60°C., treating with a little charcoal, filtering the hot solution and leaving the almost colourless filtrate to cool. The substance separated as fine white crystalline needles which, when filtered off and dried in a vacuum desiccator, had a melting point of 82-84°C. After a second purification by the same method the compound still did not have a sharp melting point (82- 84° C.) and attempts were made to obtain

a purer specimen by recrystallising from other solvents. The substance was extremely soluble in cold methyl alcohol, ethyl alcohol and acetone. It was almost insoluble in cold chloroform and toluene and moderately soluble in the hot solvents but on slowly cooling the solutions the compound was precipitated first as a colourless oil which subsequently crystallised. It dissolved readily in hot benzene from which solution it crystallised out well on cooling but the melting point of the product was only 80-82°C. The fact that the solution in hot benzene darkened fairly rapidly indicated that the compound was unstable in hot solution.

1 g. of chitose benzylphenylhydrazone was dissolved in 5 ml. of cold 96% alcohol to give a yellow coloured solution. On shaking with a little charcoal and filtering an almost colourless filtrate was obtained. When water was added dropwise to this solution a white precipitate was produced which redissolved on shaking. The addition of water was continued until on shaking the solution remained turbid. A clear liquid was again obtained when it was heated in a waterbath at 30°C. and addition of water was continued until a faintly cloudy solution was obtained at this temperature. This was divided into two equal volumes, the first being cooled rapidly in the refrigerator and the second cooled much more slowly by leaving in the waterbath at 30°C. which was left to cool spontaneously to room temperature.

From the first portion there rapidly separated a

snow white product which after filtering and drying in a vacuum desiccator melted sharply at 85°C.

From the second portion the product crystallised slowly as a mass of yellow crystals. The filtered and dried product melted at 82-84°C.

Analysis -- Microanalysis figures from three separate laboratories on 4 separately and carefully prepared samples have not given consistent results in the case of this compound

	O	H	N
found	63.18 - 64.15%	6.34 - 7.33%	7.24 - 8.79%.
$C_{19}H_{22}O_4N_2$ requires...	66.67%	6.43%	9.19%

Rotation -- $(\alpha)_D^{20°}$ = +50.25° in ethyl alcohol. c = 2.786%.

Melting point -- ... 85°C.

The crystalline p-nitrophenylhydrazone of chitose.

1.77 g. of p-nitrophenylhydrazine were ground in a mortar to a fine powder, added to 25 ml. of chitose solution and the mixture shaken vigorously for 24 hours. The solid material was then filtered off and after drying in a hot air oven at 60°C. to constant weight was shaken with 25 ml. of nitrobenzene in which p-nitrophenylhydrazine is quite soluble. The insoluble material was filtered off and washed with a little ether. The product was almost insoluble in cold water but dissolved readily in the hot solution and

crystallised out on cooling as a yellow mass consisting of small spherical clusters of fine needles. Yield of the product was 1.72 g. It melted at 175-176°C.

The compound was further purified by dissolving in hot 90% alcohol, treating the solution with a little charcoal, filtering and allowing the clear orange yellow solution to cool. The yellow crystalline product which separated was filtered off, washed with a little ether and dried in a vacuum desiccator over concentrated sulphuric acid. It had a melting point of 183-184°C. A much cleaner lemon yellow product which was obtained by recrystallising from absolute alcohol melted sharply at 185°C. No increase in melting point was obtained on further recrystallisation from absolute alcohol.

Analysis ---	C	H	N
Found	48.35%	5.00%	14.15%
$C_{12}H_{15}O_6N_3$ requires.	48.48%	5.05%	14.14%

Rotation --- $(\alpha)_D^{20^\circ} = +7.09^\circ$ in ethyl alcohol.
c = 1.410%.

Attempted estimation of the chitose content of a solution using benzylphenylhydrazine in place of 2:4dinitrophenylhydrazine.

In a series of experiments 3 ml. volumes of the chitose solution prepared as described on page 149 were diluted to

10 ml. with water and to this was added 5 ml. of a benzene solution containing 0.3 g. of benzylphonylhydrazine. Each mixture after vigorous shaking for 5 hours was filtered through a tared Gooch crucible. The crystalline hydrazone was washed with a little pure benzene and dried in an air oven at 40°C. to constant weight. Theoretical yield of the hydrazone from 3 ml. of the chitose solution = 0.4750 g.

Experiment No.	Wt. of hydrazone obtained, in grams.	Percentage yield of the hydrazone.
1	0.2700	56.8%
2	0.2837	59.7%
3	0.2136	45.0%
4	0.2223	46.8%
5	0.2316	48.8%

To the filtrate from each estimation was added a few drops of benzylphonylhydrazine and the shaking was continued for a further period of 5 hours. In no case, however, did any crystalline material separate from the mixture. The products obtained in this experiment were discoloured and all melted between 60 and 70°C. indicating the presence of a considerable quantity of impurity. Difficulty was experienced in the filtrations and washings as the extremely fine crystals formed themselves into a matted mass in each of the Gooch crucibles.

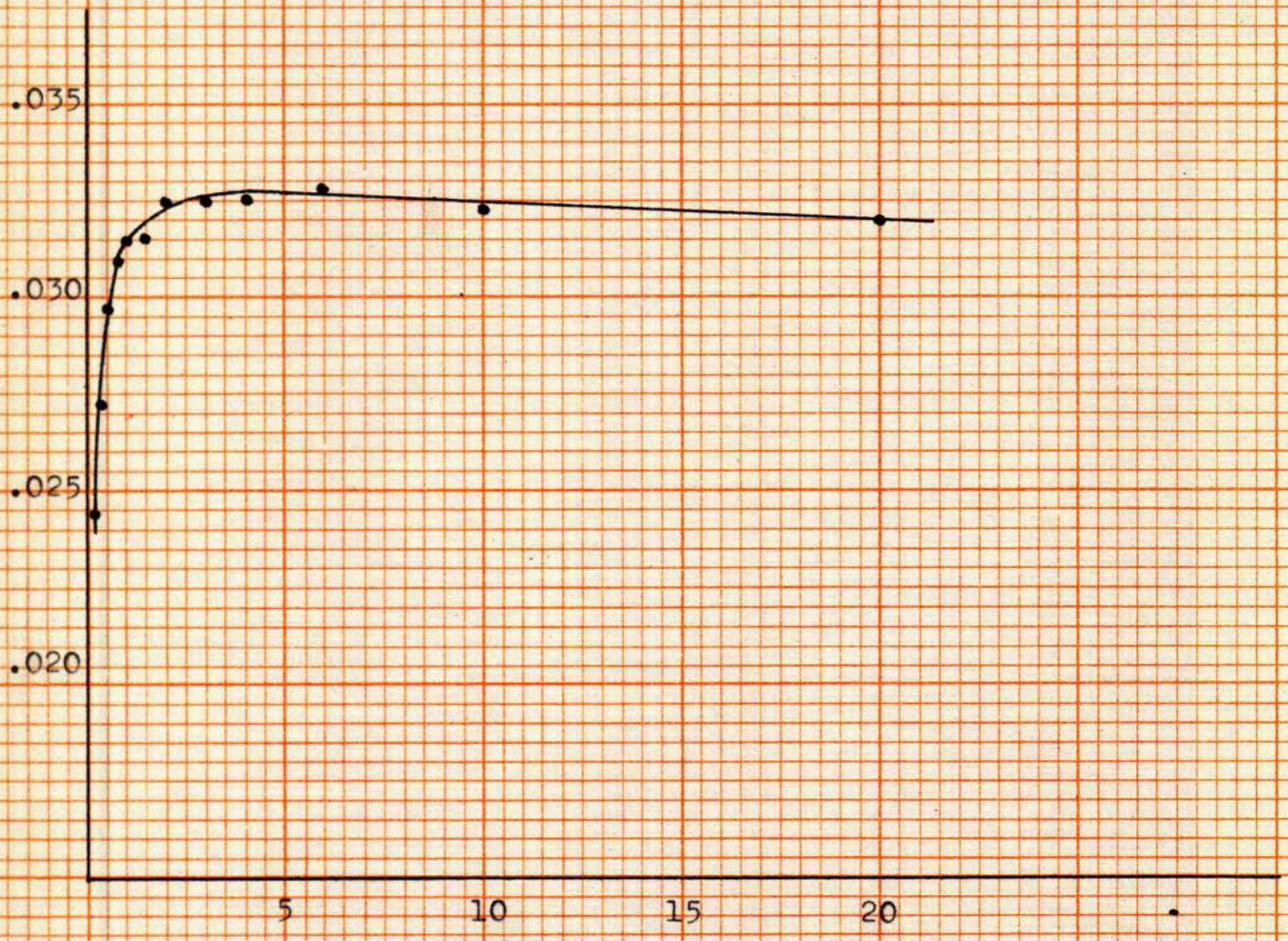
Attempted estimation of chitose in aqueous solution using p-nitrophenylhydrazine in place of 2:4dinitrophenylhydrazine.

Rate of formation of crystalline chitose p-nitrophenylhydrazone in an aqueous solution of chitose shaken with finely ground p-nitrophenylhydrazine.

In this experiment the same solution of chitose was used as that employed in the previous attempt at estimation of chitose with 2:4dinitrophenylhydrazine. It contained theoretically 0.075 g. of chitose per ml. though the actual chitose content was estimated as 52.0% of this value. An excess (about 0.1 g.) of p-nitrophenylhydrazine purified by recrystallising from 96% alcohol dried to constant weight in a vacuum desiccator and ground in a mortar to a yellow powder, was accurately weighed into a clean dry test tube and into this was pipetted 1 ml. of the chitose solution. The tube was shaken in the upright position in a mechanical shaker at such a rate that the solid hydrazine did not settle at the bottom of the tube. After shaking for a fixed period of time the contents of the tube were filtered with suction through a tared Gooch crucible fitted to an Irvine filter tube. The clear filtrate was employed repeatedly to wash out the entire solid contents of the test tube into the Gooch which was then twice washed through with 0.5 ml. of distilled water. The crucible and its solid contents was

GRAPH 7.

The rate of formation of the crystalline hydrazone on shaking a solution of chitose containing finely ground p-nitrophenylhydrazine.



Time shaken in hours.

Exp. No.	Wt. of test tube.	Wt. of test tube + hydrazine.	Wt. of the hydrazine.	Time shaken	Tare of Gooch	Wt. of Gooch + product	Wt. of product	Increase in wt. of product.
1	3.6330 g	3.7527 g	0.0997 g	10 mts.	8.0776 g	8.2016 g	.1240 g	0.0243 g
2	3.8614	3.9611	0.0997	20 "	8.7171	8.8441	.1269	0.0272
3	3.8485	3.9477	0.0992	30 "	9.1377	9.2665	.1288	0.0296
4	4.0622	4.1627	0.1005	45 "	5.9676	6.0989	.1313	0.0308
5	3.6531	3.7521	0.0990	60 "	8.0765	8.2069	.1304	0.0314
6	3.6665	3.7628	0.0963	90 "	8.6472	8.7750	.1278	0.0315
7	3.8614	3.9674	0.1060	120 "	8.7177	8.8561	.1384	0.0324
8	4.0622	4.1677	0.1055	240 "	5.9688	6.1068	.1380	0.0325
9	3.6531	3.7557	0.1026	360 "	8.0784	8.2138	.1354	0.0328
10	3.8614	3.9638	0.1024	600 "	8.7175	8.8521	.1346	0.0322
11	3.8484	3.9497	0.1013	1200"	9.1382	9.2714	.1332	0.0319

then dried to constant weight in an hot air oven at 40-50°C.

The experiment was repeated in a series of tubes, the shaking process being interrupted at varying intervals of time. The results of the experiment are indicated in the ~~following~~ opposite table, and graph 7.

From the graph it is evident that after 2 - 3 hours shaking the formation of crystalline chitose p-nitrophenylhydrazone was practically complete and that for shaking periods of more than 2 hours there was reasonably close agreement in the values obtained for the increase in weight of the product. It was noted that in the experiment in which the shaking period was prolonged for more than 6 hours a small quantity of a red oily product appeared and that this increased in amount with the period of shaking. This may have been a product of reaction between the hydrazone and the excess free p-nitrophenylhydrazine or the result of interaction between chitose decomposition products and p-nitrophenylhydrazine. A portion of this oil was lost in the process of filtration and such a loss is indicated in the results by a slight decrease in the value obtained for the increase in weight of the product.

Determination of the loss in weight of the product due to the slightly solubilities in water of p-nitrophenylhydrazine and it chitose hydrazone.

Accurately weighed quantities of each of the pure powdered dry compounds in 1 ml. of distilled water were

shaken as described in the preceding experiment, for 3 hours. Each mixture was then filtered through a tared Gooch crucible, the residue twice washed through with 0.5 ml. volumes of distilled water and dried to constant weight in an oven at 40-50°C.

No. of Experiment.	1	2	3	4
Wt. of test tube.	3.6666 g.	3.8614 g.	3.6530 g.	3.27
Wt. of tube + p-nitrophenylhydrazine.	3.7608	3.9550		
Wt. of tube + chitose p-nitrophenylhydrazone.			3.7383	3.32
Initial wt. of dry solid.	0.0942	0.0936	0.0853	0.05
Final wt. of dried product.	0.0931	0.0923	0.0844	0.04
Loss in weight of solid.	0.0011	0.0013	0.0009	0.00

Loss in weight by dissolution of p-nitrophenylhydrazine ... ... 0.0012 g.

Loss in weight by dissolution of chitose p-nitrophenylhydrazone. ... ... 0.0009 g.

Therefore the total loss in weight due to the solubilities of these two compounds in the proposed method for estimation of chitose would be 0.0021 g. which should be taken into account when calculating the percentage chitose present in a solution.

The average increase in weight obtained in the above experiment where an excess of p-nitrophenylhydrazine was shaken with 1 ml. volumes of chitose solution for periods of 2, 4 and 6 hours was 0.0326 g.

Increase in weight, allowing for solubilities ... ... 0.0347 g.

An increase in wt. of 144 g. ≡ 162 g. of chitose.

∴ " " " " 0.0347g. ≡ 0.0390 g. "

Chitose content calculated as a percentage of the theoretical value: (0.075 g. per ml.) $= \frac{.039}{.075} \times \frac{100}{1}$

$= 52.0\%$.

Value estimated by the 2:4dinitrophenylhydrazine method = 52.0%.

In all the estimations of chitose that follow the experimental technique described above was employed and a shaking period of 3 hours was adopted in every case.

<u>Examination of the effect of the presence of glucosamine hydrochloride (probably present in chitose solutions as a result of incomplete deamination) on the new method of chitose estimation.</u>

1 ml. of an aqueous solution containing 0.1077 g. of glucosamine hydrochloride was pipetted into a test tube containing an accurately weighed quantity of p-nitrophenylhydrazine and the mixture was shaken at room temperature for

3 hours. The weight of the solid material was determined by the technique described in the preceding section and the results of duplicate experiments are indicated in the following table.

No. of experiment.	1.	2.
Weight of test tube	3.2722 g.	3.8612 g.
" " " " + p-nitrophenylhydrazine.	3.3355	3.9602
Weight of p-nitrophenyl-hydrazine.	0.0633	0.0990
Tare of Gooch.	8.6449	9.2830
Wt. of Gooch+product.	8.7087	9.3827
Wt. of product.	0.0638	0.0997
Increase in wt. of product	0.0005	0.0007

The effect of the presence of comparatively small quantities of glucosamine hydrochloride in solutions of chitose would apparently be negligible.

<u>Examination of the effect of the possible presence of glucose and/or mannose in solutions of deaminated glucosamine hydrochloride on the new chitose estimation method.</u>

A solution of glucose was made up by dissolving 0.75 g. in 10 ml. of water. A second solution containing the same concentration of mannose was also prepared. The chitose estimation method was carried out on 1 ml. of each of these solutions and the following results were obtained.

	Glucose solution	Mannose solution
Wt. of test tube.	3.2719 g.	3.8609 g.
Wt. of test tube + p-nitrophenylhydrazine.	3.3990	3.9772
Wt. of the hydrazine.	0.1271	0.1163
Tare of Gooch.	5.9666	8.0780
Wt. of Gooch+product.	6.0916	8.1926.
Wt. of product.	0.1250	0.1146
Loss in wt. of solid.	0.0012 g.	0.0017 g.

The presence of glucose or mannose in chitose solutions would apparently have no appreciable effect on the determination of chitose by this method.

EXAMINATION OF THE STABILITY OF CHITOSE.

The effect of heat on an aqueous solution of chitose.

About 10 ml. of the chitose solution calculated to contain 52.0% of the theoretical amount was transferred to a test tube and the level of the liquid at room temperature marked on the tube. This was immersed in a water bath at 90°C. and left at that temperature for 1 hour. The liquid which had appreciably darkened in colour was allowed to cool to room temperature when it was made up to the original volume with water.

Rotation of the solution in ½ dm. tube at 20°C. .... +0.80°

Rotation of original solution in ½ dm. tube at 20°C..... .... +1.06°

Chitose content of the solution and percentage decomposition —

Estimation.	1	2	3
Wt. of test tube.	4.0622 g.	3.6530 g.	3.8614 g.
Wt. of test tube + p-nitrophenylhydrazine.	4.1615	3.7538	3.9632
Wt. of the hydrazine.	0.0993	0.1008	0.1018
Tare of Gooch.	5.9684	8.0778	5.9677
Wt. of Gooch+product.	6.0931	8.2044	6.0949
Wt. of product.	0.1247	0.1266	0.1272
Increase in wt. of solid	0.0254	0.0258	0.0254
Wt. of chitose in 1 ml. solution.	0.0309	0.0314	0.0309

Average value ..... 0.0311 g.

Weight of chitose in 1 ml. of original solution. .... 0.0390 g.

Percentage decomposition. ... ... 20.3%.

Effect of aeration on a chitose solution.

10 ml. of the chitose solution was vigorously aerated with atmospheric air at room temperature for 3 hours and then made up to the original volume with a little distilled water.

The rotation of the solution remained unchanged at $+1.06^{\circ}$ in $\frac{1}{2}$ dm. tube.

Chitose content of the resulting solution .. 0.0389 g. per ml.

" " " " original solution ... 0.0390 g. per ml.

The effect of aeration is negligible.

Effect of evaporation of chitose solution in vacuo at 40°C. to a viscous syrup.

20 ml. of the chitose solution was pipetted into a distilling flask and the water removed in vacuo at 40°C. until a viscous yellow syrup was obtained. This was dissolved in water and the solution again made up to 20 ml. The chitose content of the resulting solution was estimated as 0.0254 g./ml.

Chitose content of original solution .... 0.0390 g./ml.
Percentage decomposition ... ... 34.9%.
Rotation of the solution in ½ dm. tube... +1.04°
Rotation of the original solution in ½ dm. tube... +1.05°

It is noteworthy that in this experiment where almost 35% of the chitose suffered decomposition there was only a small difference between the rotatory powers of the original and final solutions whereas the change in rotation of a solution in which chitose was decomposed to the extent of 20.3% by heating to 90°C. for 1 hour was relatively great. It is apparent that no indication of the actual chitose content of a solution could be obtained from its rotatory power.

Effect of evaporating the chitose solution in vacuo at 40°C. to a moderately mobile syrup.

20 ml. of the chitose solution pipetted into a distilling flask was evaporated under diminished pressure at 40°C. to a fairly mobile syrup (3-4 ml.). This was washed out with distilled water into a 20 ml. graduated flask and the solution made up to the graduation mark.

Chitose content of the final solution ... 0.0353 g./ml.
Chitose content of the original soln. ... 0.0390 g./ml.
Percentage decomposition .... 8.7%.

The loss in this case is not nearly so great as in the

case where the evaporation was continued to the formation of a viscous syrup. Decomposition of chitose appears to be extensive only when the solution becomes extremely concentra or almost dry.

Effect of evaporation of the chitose solution in vacuo at 0°C. to a stiff syrup.

20 ml. of the chitose solution in a shallow beaker was placed in a desiccator over concentrated sulphuric acid. The desiccator was evacuated by the water pump and left in the refrigerator at 0°C. After 4 days the liquid was reduced to a stiff syrup which dissolved in water and the solution made up to 20 ml. in a graduated flask.

Chitose content of the resulting solution ...	0.0325 g./
" " " " original solution ...	0.0390 "
Percentage decomposition	16.7%.

Effect of evaporation in vacuo at 0°C. of a series of chitose solutions varying in acidity.

A volume of 10 ml. of the chitose solution was pipetted into each of 6 small glass evaporating dishes. To solution 1 was added about 0.2 g. of sodium acetate making it neutral or very faintly alkaline to litmus paper. To solution 2 was added only ½ of this amount of sodium acetate and 1 drop of 10% acetic acid, thus making it slightly acid to litmus but alkaline to congo red paper.

No additions were made to solution 3.

To solution 4 was added 0.5 ml. of glacial acetic acid giving a chitose solution in approximately 5% acetic acid which was slightly acid to congo red paper.

1 ml. of glacial acetic acid was added to solution 5 making it acid to congo red paper. The solution contained approximately 10% acetic acid.

To solution 6 was added 0.3 ml. of concentrated hydrochloric acid making it strongly acid to congo red peper. It contained approximately 1% hydrochloric acid.

Each solution was placed over concentrated sulphuric acid in a desiccator which was then evacuated by the water pump and left in the refrigerator at 0^{o}C. for 4 days when each solution was reduced to a viscous syrup. In each case the syrup was dissolved in water and the solution made up to 10 ml. in a graduated flask. These solutions were greenish yellow in colour except in the case of solution 6 which was dark brown, indicating that extensive decomposition had taken place. It was also noted during the subsequent estimations of chitose in these solutions that whereas solutions 1-5 gave solid crystalline products with p-nitrophenylhydrazine, the product from solution 6 contained a large quantity of a dark oily material.

Chitose contents of the final solutions and percentage decomposition in each case.

Solution No.	1	2	3	4	5
Chitose content (g./ml.)...	0.0291	0.0327	0.0318	0.0325	0.0317
Percentage decomposition.	25.4%	16.2%	18.5%	16.7%	18.7%

The results of this experiment indicate that chitose is more unstable in slightly alkaline solution than in a slightly acid medium. Its stability appears to be approximately the same in solutions of acidity between that of a 10% acetic acid solution and that of a very faintly acid solution. Decomposition appears to be extremely rapid, however, in dilute hydrochloric acid solution. In the case of solution 6 there was an actual loss in the weight of solid p-nitrophenylhydrazine when attempts were made to estimate its chitose content and it appeared that extensive destruction of the chitose molecule had occurred.

DEAMINATION OF GLUCOSAMINE HYDROCHLORIDE SOLUTIONS.

Preparation of chitose solution by deamination of glucosamine hydrochloride with sodium nitrite in a solution slightly acidified with acetic acid.

The preceding investigation into the stability of chitose suggested that a higher yield of chitose might be obtained by deamination of glucosamine hydrochloride in a slightly more acid medium. Accordingly 2.5 g. of glucosamine hydrochloride was dissolved in 20 ml. of distilled water and to this solution cooled in an ice bath was added 0.81 g. (1 mol. of solid sodium nitrite. The liquid was agitated until complete dissolution had occurred. This solution was faintly acid to litmus paper and alkaline to congo red paper. 1 drop of glacial acetic acid was added making the solution faintly acid to congo red paper. This was left for 4 days in the refrigerator then for a further period of 4 days at room temperature until evolution of nitrogen from the solution had practically ceased. The solution was made up to 25 ml. in a graduated flask. Estimated chitose content of the solution was 0.0512 g./ml. Percentage yield (theoretical amount 0.075 g./ml.) .. 68.3%.

Yield of chitose previously obtained by deamination of glucosamine hydrochloride solution not acidified with acetic acid was ... 52.0%.

The action of dry methyl alcoholic hydrochloric acid on chitose.

The solution prepared in the preceding experiment containing a high yield of chitose was evaporated to a stiff syrup in a vacuum desiccator over concentrated sulphuric acid at 0°C. The syrup was dissolved in about 10 ml. of dry methyl alcohol and the solution filtered from the insoluble inorganic matter (mostly sodium chloride). To the clear light yellow solution was added 0.5 ml. of a methyl alcohol solution containing 20% of dry hydrochloric acid and the resulting liquid made up to 25 ml. with pure methyl alcohol. The solution thus contained 0.5% hydrochloric acid. The following rotational changes were observed when the solution was examined from time to time in the polarimeter.

Time after solution was made up.	Rotation at room temperature in 1 dm. tube.
10 mts.	+2.50°
1 hour.	+2.54°
2 hours	+2.57°
4 "	+2.61°
6 "	+2.63°
8 "	+2.65°
12 "	+2.67°
24 "	+2.60°
30 "	+2.53°
36 "	+2.48°
48 "	+2.48°

After 24 hours it was noted that a white solid had separated from the solution and this gradually increased in amount over the next 24 hours. The solid material was filtered off and identified as glucosamine hydrochloride. This product was probably produced from free glucosamine present as a result of incomplete deamination in the preparation of chitose. The observed changes in rotation recorde above cannot therefore be taken as an indication of the formation of methyl chitoside or chitose dimethylacetal in the solution.

Examination of the extent of deamination of glucosamine hydrochloride under varying experimental conditions.

Preparation of standard solutions of glucosamine hydrochloride and sodium nitrite.

Both substances were purified by recrystallising from water.

10.7750 g. of glucosamine hydrochloride was accurately weighed out, dissolved in distilled water and the solution made up to 50 ml. in a graduated flask.

3.4500 g. of sodium nitrite in 50 ml. of aqueous solution was similarly prepared.

These solutions contained equivalent quantities of the reagents and a complete deamination of 2 ml. of the glucosamine hydrochloride solution should result in the evolution

of 44.8 ml. of nitrogen at N.T.P.

In the following series of experiments the reaction flask was attached to a Lunge nitrometer and the extent of deamination calculated from the volume of nitrogen evolved.

(1) 2 ml. of glucosamine hydrochloride solution and 2 ml. of sodium nitrite solution were pipetted into the reaction flask which was left at room temperature throughout the course of the deamination. The rate and extent of deaminatio is indicated in the following table.

Reaction time, in hours.	Ml. of nitrogen evolved at room temperature.
1	4.4
2	10.4
6	24.2
23	36.1
29	38.7
45	39.7
72	39.8
96	39.8

Room temperature at the final reading .. 19°C.
Atmospheric pressure at " " .. 773 mm.
Final volume of nitrogen at N.T.P. .. 37.8 ml.
Theoretical volume of nitrogen at N.T.P. .. 44.8 ml.
∴ Percentage deamination. ... ... 84.4%.

---

(2) To 2 ml. of the glucosamine hydrochloride solution in the deaminating flask was added 2 ml. of sodium nitrite solution and 0.2 ml. of 10% acetic acid.

Reaction time.	Ml. nitrogen evolved at room temperature.
5 mts.	12.0
10 "	19.2
20 "	27.2
1 hour	31.6
1½ "	32.6
2 "	33.0
6 "	35.0
24 "	35.6
48 "	35.7
72 "	35.6

Room temperature at the final reading ... 16°C.
Atmospheric pressure at the " " ... 768 mm.
Final volume of nitrogen at $\overset{\text{vv}}{\text{N.T.P.}}$ ... 34.0 ml.
Percentage deamination ... ... 75.9%.

In this case it was noted that the rate of deamination was much more rapid than in the case of the unacidified solut although the extent of deamination was more incomplete. On addition of a small amount of sodium nitrite to the final solution evolution of nitrogen recommenced. It therefore seemed probable that from such an acidified deaminating mixture there would be a considerable loss of nitrous acid passing off in the evolved nitrogen.

---

(3) To 2 ml. of the solution of glucosamine hydrochloride in the deaminating flask was added 2.5 ml. (1½ mol.) of sodium nitrite solution and 0.1 ml. of 10% acetic acid.

Reaction time.	Ml. of nitrogen evolved at room temperature.
5 mts.	10.4
10 "	20.1
20 "	28.1
30 "	31.4
1 hour 10 mts.	35.8
2 hours 25 "	37.9
7 "	39.8
30 "	42.2
48 "	43.0
72 "	43.0

Room temperature at the final reading ... 12^{0}C.
Atmospheric pressure at " " ... 762 mm.
Final volume of nitrogen at N.T.P. ... 41.3 ml.
∴ Percentage deamination. ... 93.9%.

---

(4) The preceding experiment was repeated with the exceptions that 0.2 ml. of 10% acetic acid was added and that the solution was kept throughout the reaction in a ice salt bath at -2^{0} to -3^{0}C.

Reaction time.	Ml. of nitrogen evolved measured at room temperature and atmospheric pressure.
10 mts.	9.3
30 "	18.2
1 hour	31.0
2 hours	37.6
5 "	43.1
7½ "	44.3
10 "	45.8
24 "	45.6
48 "	45.6

Room temperature at final reading ... 18°c.

Atmospheric pressure at final reading .. 760 mm.

Final volume of nitrogen at N.T.P. .. 43.1 ml.

Percentage deamination.. ... . 96.2%.

---

(5) The preceding experiment was repeated using 0.3 ml. instead of 0.2 ml. of 10% acetic acid.

Reaction time.	Ml. of nitrogen evolved.
20 mts.	17.4
38 "	24.6
1 hour 15 mts.	33.4
2 hours 10 "	39.1
2 " 45 "	41.1
5 " 0 "	44.2
6 " 45 "	45.5
8 " 0 "	46.4
26 " 0 "	47.4
48 " 0 "	47.4

Room temperature at the final reading	...	18°C.
Atmospheric pressure at " "	...	758 mm.
Volume of nitrogen evolved at N.T.P.	...	44.3 ml.
∴ Percentage deamination.	...	98.9%.

Deamination of glucosamine hydrochloride solution with silver nitrite.

(6) To 2 ml. of the standard glucosamine hydrochloride solution was added 2 ml. of water and 0.62 g. of silver nitrite. Deamination was carried out according to the method of Zechmeister and Toth at 0°C. for 1 hour, then at room temperature. Throughout the deamination the reaction flask was shaken from time to time.

Reaction time.	Ml. of nitrogen evolved.
45 mts.	8.2
1 hour 40 mts.	19.1
2 hours 0 "	21.1
3 " 45 "	26.8
8 " 15 "	33.1
23 " 15 "	36.5
30 " 0 "	36.6
48 " 0 "	36.6

Room temperature at the final reading	...	20°C.
Atmospheric pressure at " "	...	770 mm.
Volume of nitrogen evolved at N.T.P.	...	34.5 ml.
Percentage deamination.	...	77.0%.

(7) To 2 ml. of the glucosamine hydrochloride solution was added 1.9 ml. of water, 0.1 ml. of 10% acetic acid and 0.62 g. of silver nitrite. The deamination flask was shaken from time to time and kept at 0°C. in an ice bath throughout the reaction.

Reaction time.	Ml. of nitrogen evolved.
10 mts.	3.2
20 "	6.6
30 "	9.9
50 "	15.1
2 hours	25.3
3 "	29.2
5 "	31.7
6 "	32.6
24 "	35.7
48 "	35.8

Room temperature at the final reading ... 15°C.
Atmospheric pressure at " " ... 756 mm.
Volume of nitrogen evolved at N.T.P. ... 33.8 ml.
Percentage deamination. ... ... 76.3%.

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(8) The preceding experiment was repeated using 0.2 ml. of 10% acetic acid and 1.8 ml. of water instead of 0.1 ml. of 10% acetic acid and 1.9 ml. of water.

Reaction time.	Ml. of nitrogen evolved.
10 mts.	8.8
20 "	14.8
40 "	23.4
1 hour.	28.4
2 hours	34.7
3 "	37.3
5 "	39.0
24 "	40.4
48 "	40.3

Room temperature at the final reading ... 14°C.
Atmospheric pressure at " " ... 757 mm.
Volume of nitrogen at N.T.P. .... 38.2 ml.
Percentage deamination. ... . 85.3%.

(9) The above experiment was again repeated using 1.7 ml. of water and 0.3 ml. of 10% acetic acid solution.

Reaction time.	Ml. of nitrogen evolved.
20 mts.	17.5
1 hour.	30.9
2 hours	36.0
3 "	38.3
6 "	40.1
12 "	41.0
24 "	41.3
48 "	41.5

Room temperature at final reading ... 15°C.
Atmospheric pressure at " " ... 758 mm.
Volume of nitrogen at N.T.P. ... ... 39.2 ml.
Percentage deamination. ... ... 87.5%.

(10) The experiment was repeated with 0.4 ml. of 10% acetic acid and 1.6 ml. of water.

Reaction time.	Ml. of nitrogen evolved.
20 mts.	21.5
1 hour.	34.0
2 hours	39.9
3 "	41.9
5 "	42.6
8 "	42.6
24 "	42.8

Room temperature at final reading ... 17°C.

Atmospheric pressure at final reading ... 755 mm.

Volume of nitrogen at N.T.P. .... 40.0 ml.

Percentage deamination. ... . 89.3%.

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(11) The experiment was repeated with 0.8 ml. of 10% acetic acid and 1.2 ml. of water.

Reaction time.	Ml. of nitrogen evolved.
15 mts.	15.8
30 "	28.5
1 hour	34.4
2 hours	40.1
6 "	41.5
24 "	42.1
48 "	42.3

Room temperature at the final reading ... 14°C.

Atmospheric pressure at " " ... 760 mm.

Volume of nitrogen at N.T.P. .... 40.2 ml.
Percentage deamination. ... . 89.7%.

---

(12) To the deaminating flask containing 0.62 g. (2 mols.) of finely powdered silver nitrite was added 1.9 ml. of water, 0.1 ml. of 10% hydrochloric acid solution and 2 ml. of the standard glucosamine hydrochloride solution. The flask was immersed in an ice bath and agitated from time to time throughout the deamination.

Reaction time.	Ml. of nitrogen evolved.
15 mts.	5.8
30 "	12.0
1 hour	20.8
2 hours	28.4
3 "	37.0
4 "	39.9
10 "	43.2
48 "	45.6
72 "	45.7

Room temperature at the final reading ... 15°C.
Atmospheric pressure at " " ... 753 mm.
Volume of nitrogen at N.T.P. .... 43.6 ml.
Percentage deamination. ... . 97.3%.

PREPARATION AND PROPERTIES OF A SOLUTION OF HIGH CHITOSE CONTENT.

Chitose content of solutions obtained by practically complete deamination of glucosamine hydrochloride at low temperature with (a) silver nitrite and (b) sodium nitrite.

(a) The deaminated mixture obtained in experiment (12) of the preceding section was filtered with suction and the silver residues washed through several times with a few drops of distilled water. To the clear colourless solution was added drop by drop N/10 hydrochloric acid solution until no further precipitate of silver chloride was obtained. Agai the solution was filtered and washed through with distilled water. After strongly aerating for half an hour to remove nitrous acid the solution was made up to a volume of 10 ml. in a graduated flask.

Chitose content = 74.1% of the theoretical amount.

(b) The solution obtained in experiment (5) of the preceding section, prepared by almost complete deamination of glucosamine hydrochloride with an excess (1½ mols.) of sodium nitrite and a quantity of acetic acid equivalent to the excess of nitrite added, was freed from nitrous acid by addition of 1 ml. of 10% acetic acid and vigorous aeration of the resulting solution at room temperature for

half an hour. The solution was then made up to 10 ml. in a graduated flask. It contained 1% acetic acid which it was considered would appreciably affect the solubility of p-nitro-phenylhydrazine and consequently for the estimation of chitose in this solution a correction factor was determined by shaking a quantity of the finely ground solid hydrazine for 3 hours in 1 ml. of 1% acetic acid solution and finding the loss in weight of solid material.

Estimated loss in weight of p-nitro-phenylhydrazine, when shaken with 1 ml. of 1% acetic acid solution .... 0.0026 g.

Estimated chitose content of the solution.. 93.7% of the theoretical quantity.

Preparation of chitose solution from pure chitose 2:4dinitro-phenylhydrazone.

(1) To 0.2 g. of pure crystalline chitose 2:4dinitrophenyl-hydrazone in a stoppered bottle was added 50 ml. of 1% acetic acid and a solution of 5 ml. of benzaldehyde in 10 ml of benzene and the mixture vigorously shaken at room temperature for 48 hours. During this period the yellow crystals of chitose 2:4dinitrophenylhydrazone completely dissolved and were replaced by a red crystalline mass. The latter, seperated by filtration and recrystallised from 90% alcohol had a melting point of 234°C. identical with that of a specimen of benzylidene 2:4dinitrophenylhydrazone

prepared directly from benzaldehyde and 2:4dinitrophenylhydrazine. There was no depression of melting point on mixing the two samples.

The filtrate from the reaction mixture was transferred to a separating funnel and the benzene layer discarded. The aqueous layer which was only very light yellow in colour was extracted three times with 20 ml. volumes of ether to remove benzoic acid and the resulting aqueous solution evaporated *in vacuo* at 25°C. to a small volume. This was made up to 5 ml. and the chitose content estimated.

Theoretical quantity of chitose obtainable from 0.2 g. of chitose 2:4dinitrophenylhydrazone ..0.094

∴ 1 ml. solution contains theoretically 0.0189 g. chitose.

Estimated chitose content of the solution, 0.0140 g./ml.

∴ Yield of chitose, 73.8% of the theoretical amount.

---

(2) 0.5 g. of chitose 2:4dinitrophenylhydrazone was dissolved in 10 ml. of benzaldehyde. The clear solution was left in the refrigerator for 7 days, during which time the crystalline benzylidene 2:4dinitrophenylhydrazone gradually separated from solution. At the end of this period a test portion of the solution was withdrawn and shaken with about 1 ml. of water and 1 ml. of ether. The aqueous layer remained practically colourless indicating that no chitose 2:4dinitrophenylhydrazone was present and

that therefore the reaction was practically completed. (A freshly prepared solution of chitose 2:4dinitrophenylhydrazon in benzaldehyde when shaken with water and ether mixture imparted an orange yellow colour to the aqueous layer). The reaction mixture was vigorously shaken with 10 ml. of water and 10 ml. of ether and filtered with suction. The aqueous layer was removed in a separating funnel, shaken three times with small volumes of ether to remove any benzoic acid and made up to 15 ml. in a graduated flask.

Theoretical chitose content of the solution .. 0.0158 g. m.

Chitose content determined by estimation ..... 0.0087 g. m.

Percentage yield of chitose ..... 54.8%.

Attempted preparation of d-arabinosazone and d-glucosazone from chitose solution prepared from pure chitose 2:4dinitrophenylhydrazone.

A few crystals of sodium acetate were dissolved in 5 ml. of the chitose solution prepared in the preceding experiment and to this was added 0.5 ml. of phenylhydrazine and just sufficient 30% acetic acid to completely dissolve the hydrazine. No crystalline material separated from the solution when it was left for 3 hours at room temperature, but on heating in a boiling waterbath for 2 hours clusters of light yellow crystalline material, resembling glucosazone when examined under the microscope, were obtained. The

product was filtered off and left in air at room temperature to dry. 0.06g of the crude product were thus obtained. It was purified by recrystallising from 96% alcohol, filtering, washing the product with ether and drying in a vacuum desiccator over concentrated sulphuric acid.

Melting point of the purified product ... 208°C.

Mixed melting point with pure d-glucosazone .. 208°C.

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d-arabinosazone.

5 ml. of the same chitose solution was acidified with a drop of 2N hydrochloric acid and the solution aerated vigorously at room temperature with atmospheric air. To this was then added 0.5 ml. of phenylhydrazine and just sufficient 30% acetic acid to dissolve the hydrazine completely. The liquid was left at room temperature for 3 hours but no crystalline material separated from solution.

These experiments indicate that d-glucosazone is a reaction product of chitose and phenylhydrazine and that the d-glucosazone prepared from chitose solutions formed by deamination of the glucosamine hydrochloride is not solely a reaction product of phenylhydrazine and undeaminated glucosamine. d-arabinosazone on the other hand, would appear to be derived not from chitose or one of its

decomposition products but from some secondary reaction product formed from glucosamine hydrochloride in the deamination process.

Preparation of chitose solution.

A 500 ml. distilling flask containing a solution of 15.0 g. of glucosamine hydrochloride in 100 ml. of water was cooled in an ice-salt bath until a quantity of ice had formed in the liquid. To this was added 6.03 g. (1½ mols of sodium nitrite which was washed down from the sides of the flask with 35 ml. of ice cold water and the contents of the flask agitated to accelerate complete dissolution of the salt. The flask was again placed in the ice-salt bath until freezing commenced at approximately -3°C. 1 ml. of glacial acetic acid added to the mixture at this point distinctly increased the rate of nitrogen evolution. The solution was maintained throughout the deamination just above its freezing point (-2° to -3°C.). In the initial stages the heat of reaction was such that it was found necessary to have the bath temperature at -8° to -10°C. in order to maintain this condition but as the vigour of the reaction subsided over a period of 2 to 2½ hours the bath temperature was gradually raised to -3°C. At this point the bath and reaction flask were transferred to the refrigerator and left for 24 hours. 1.5 ml. of glacial acetic acid was added to the resulting light yellowish green solution which was then vigorously aerated

at 20°C. for 30 minutes to remove nitrous acid. The solution was finally made up to 150 ml. in a graduated flask at 15°C. The theoretical chitose content of the solution was 0.0752 g. ml.

Estimated chitose content of the solution ... 0.0702 g./ml.
Percentage of theoretical yield ... . 93.4%.
Rotation of the solution at 15°C. in a 1 dm. tube.. +2.44°

∴ approximately $(\alpha)_D^{15^\circ}$ = +34.6° in 1% acetic acid. c = 7.02%.

Reactions of the chitose solution.

It rapidly reduced Fehling's solution at room temperature.

The solution gave an intense Molisch' reaction.

The addition of 30% caustic soda produced a dark yellow colour in the chitose solution.

It gave a positive Seliwanoff reaction.

It did not restore the colour to Schiff's reagent.

Approximate estimation of the quantity of arabinosone produced in the preparation of chitose solution by this method.

To 10 ml. of the chitose solution was added 1 g. of phenylhydrazine in 3 ml. of benzene and the mixture was

shaken vigorously for 1 hour. The crystalline material, most of which separated in the first few minutes of the shaking period, was filtered with suction into a tared Gooch crucible, cleaned by washing with a few ml. of benzene and dried in a hot air oven at 40-50°C. to constant weight.

Weight of the crystalline product ... ... 0.0177 g.
Melting point ... ... 149-152°C.

No depression of melting point was observed after it had been mixed with a specimen of d-arabinosazone.

328 g. of d-arabinosazone ≡ 148 g. of d-arabinosone.

∴ 0.0177 g. " ≡ 0.0080 g. "

i.e., wt. of d-arabinosone in 10 ml. of chitose solution = 0.008 g.

Theoretical chitose content of 10 ml. solution = 0.752 g.

It is apparent from these figures that the relatively small amount of d-arabinosone present in a solution of chitose prepared by the above method would not appreciably effect the estimation of chitose by the p-nitrophenyl-hydrazine method.

In view of the fact that the solution in which chitose was estimated in this case contained 1% acetic acid it was considered desirable to examine the effect of the presence of glucose and mannose (possibly formed in small amount by the deamination of glucosamine hydrochloride) in 1% acetic acid solutions on the chitose estimation method.

0.7 g. of glucose was dissolved in 1% acetic acid and the solution made up to 10 ml. in a graduated flask. A solution containing the same concentration of mannose in 1% acetic acid was also prepared. The estimation method was carried out on these two solutions, the results of which are indicated in the following table.

	Glucose solution	Mannose solution
Wt. of test tube.	4.0157 g.	4.0217 g.
Wt. of test tube + hydrazine.	4.1322	4.1438
Wt. of hydrazine.	0.1165	0.1221
Tare of Gooch.	8.7158	9.1369
Wt. of Gooch+product.	8.8243	9.2517
Wt. of solid.	0.1085	0.1148
Decrease in wt. of solid.	0.0080	0.0073

In both cases the decrease in weight cannot be fully accounted for by the solubility of the hydrazine in 1% acetic acid and is probably due to some condensation with the formation of small amounts of glucose p-nitrophenyl-hydrazone and mannose p-nitrophenylhydrazone respectively which remain in solution. It is apparent, however, from the result that any relatively small amounts of glucose or mannose which could be present in chitose solution would not interfere with the estimation of chitose to any appreciable extent.

Extent of chitose decomposition resulting from evaporation of its solution in vacuo at 20-25°C. to a mobile syrup and removal of most of the remaining water by acetone extraction.

20 ml. of the chitose solution prepared by the improved method described in the preceding section were evaporated under diminished pressure at 23-25°C. to a volume of 2-3 ml. The remaining water was almost completely removed by thoroughly extracting the syrup with five 10 ml. volumes of cold dry acetone giving an almost solid amorphous mass. Most of the acetone was decanted off and the remainder removed by evacuating the flask at 20°C. first at the water pump then for 10 minutes under high vacuum. The resulting mass was dissolved in water and the solution made up to 20 ml. in a graduated flask.

The combined acetone extracts evaporated under diminishe pressure at 20°C. gave only a very small quantity of a syrupy material from which the last traces of acetone were removed under high vacuum at 20°C. The residue was then dissolved in water and the solution made up to 20 ml. in a graduated flask.

The chitose contents of the two solutions thus prepared were estimated in the usual manner.

Chitose content of the solution containing the acetone insoluble material	0.0644 g./m
Chitose content of the original solution ..	0.0702 g./m
Loss of chitose involved in reducing the solution to an almost dry amorphous mass ..	8.3%.

Theoretical chitose content of original solution ...
0.0752 g./ml.

Therefore the yield of almost dry amorphous chitose thus obtained from glucosamine hydrochloride after some loss in the deamination of the solution and a further loss in evaporation and water extraction was 85.6% of the theoretical amount.

The chitose estimation carried out on the solution containing the acetone soluble extract resulted in a loss of weight of p-nitrophenylhydrazine amounting to 0.0012 g. which is that representing the solubility of p-nitrophenylhydrazine in water. Chitose is therefore practically completely insoluble in acetone.

THE CONDENSATION OF CHITOSE WITH METHYL ALCOHOL.

Action of methyl alcohol on chitose in neutral solution.

120 ml. of the chitose solution prepared as described on page 188 was evaporated *in vacuo* at 20-25°C. to a volume of 15-20 ml. The resulting mobile liquid was thoroughly extracted with five 50 ml. volumes of dry acetone thus forming a semisolid mass of chitose. As much as possible of the acetone was decanted off and the remainder removed at 20°C. under diminished pressure. To this material was added 20 ml. of dry methyl alcohol and the mixture shaken until the chitose had completely dissolved. The remaining insoluble salts, mainly sodium chloride, were removed by rapid filtration, the residue being washed with a few ml. of dry methyl alcohol. The filtrate was made up to 200 ml. with dry methyl alcohol giving a light yellow coloured solution neutral to moist litmus paper. The rotation of the solution at room temperature in a 1 dm. tube 20 minutes after addition of methyl alcohol to the amorphous chitose was +1.57°. After standing 24 hours at room temperature there was only a slight increase in this value to +1.59° whic over a period of 5 days, remained unchanged.

From the observed rotations it was not suspected that condensation had taken place to any appreciable extent. However, when Fehling's reaction was carried out in the cold with 2 ml. of Fehling's solution and 0.5 ml. of the

methyl alcoholic chitose solution immediately after the solution was made up and again after an interval of 5 days a considerably smaller amount of cuprous oxide was deposited in the second case indicating that a considerable proportion of the chitose had condensed with methyl alcohol.

10 ml. of the resulting methyl alcoholic chitose solution in a small distilling flask was rendered distinctly alkaline to moist litmus paper by shaking with a small quantity (0.01 g.) of anhydrous sodium carbonate to prevent hydrolysis as far as possible during the subsequent evaporation process. On evacuating the flask with the water pump, evaporation proceeded rapidly causing the solution to become intensely cold and a layer of ice to be deposited on the outside of the distilling flask. Under such conditions it was hoped that hydrolysis of any chitose - methyl alcohol condensation product, present in the solution would be reduced to a minimum. After 2 hours when the solution had been reduced to a viscous syrup the flask was immersed in a water bath at 30-35°C. whereupon the mass swelled up and in 30 minutes was obtained as a very light yellow brittle amorphous solid which did not decrease in weight when left *in vacuo* at this temperature for a further period of 30 minutes. The product adhered rigidly to the bottom of the distilling flask but was readily broken up and reduced to a powder with a dry glass rod.

Methoxyl content of the dry product ... 6.8%.
Theoretical methoxyl content of methyl chitoside.. 17.6%.

The solid amorphous material thus prepared rapidly reduced Fehling's solution at room temperature. It was practically insoluble in both acetone and ethyl acetate. It dissolved readily in water, absolute alcohol and methyl alcohol but was almost completely insoluble in isopropyl alcohol, butyl alcohol and amyl alcohol. The difference in solubility between the suspected methoxyl derivative and free chitose was apparently too small to allow a separation to be carried out.

The effect of the presence of sodium acetate and sodium carbonate on the methoxyl content of the solid was tested by neutralising a solution of 1% acetic acid in dry methyl alcol with a slight excess of anhydrous sodium carbonate, evaporat the solution at low temperature (below 0°C.) to a white soli and drying the material to constant weight in vacuo at 30° C. The methoxyl content of the material thus prepared was 4.6%. Since the amorphous solid obtained from the methyl alcoholic chitose solution contained only about 10% of sodium acetate and sodium carbonate the effect of the presence of these sal on the methoxyl content would be only slight.

Action on chitose of methyl alcohol acidified with acetic ac

To the remainder of the above solution of chitose in

methyl alcohol was added 0.05 g. of glacial acetic acid making it slightly acid to moist litmus paper. The rotation of this solution in a 1 dm. tube at room temperature remained unchanged at $+1.59^{0}$. After 3 days at room temperature 10 ml. of the solution was removed, made definitely alkaline to litmus paper by shaking with a little anhydrous sodium carbonate and evaporated as described above to a dry amorphous mass.

Methoxyl content of the solid product ... 10.2%.

To the remaining methyl alcoholic chitose solution (180 ml.) was added a further 0.05 g. of glacial acetic acid to give a solution containing approximately 0.05% acetic acid. After 3 days at room temperature there was still no observable change in the rotatory power of the solution nor was there any apparent decrease in the reducing power towards Fehling's solution as judged from the deposit of cuprous oxide obtained at room temperature from 2 ml. of Fehling's solution and 0.5 ml. of chitose solution. 5 ml. of the solution made alkaline by shaking with 0.05 g. of anhydrous sodium carbonate was evaporated to a dry solid at low temperature.

Methoxyl content of the solid .... 10.4%.

To 9.9 ml. of the remaining methyl alcoholic chitose solution containing approximately 0.05% acetic acid was added 0.1 g. of glacial acetic acid giving a solution

containing slightly more than 1% acetic acid. The rotation of this solution (+1.58° in a 1 dm. tube) remained unaltered over a period of 24 hours at room temperature. 5 ml. of the liquid were shaken with successive small additions of anhydrous sodium carbonate until it became distinctly alkaline to moist litmus paper whereupon it was evaporated *in vacuo* to a dry solid as previously described.

Methoxyl content of the dry product ... 10.5%.

The fact that, in the three preceding experiments, the methoxyl content of the isolated solid did not appreciably increase as the amount of acetic acid in the solution was increased could be accounted for by postulating attainment of equilibrium in the system:-

Chitose + methyl alcohol ⇌ Chitose-methyl alcohol condensation product. } + water.

The remaining 5 ml. of the solution containing 1% acetic acid was evaporated *in vacuo* to a dry solid at low temperature without first neutralising with anhydrous sodium carbonate.

Methoxyl content of the solid product ... 7.4%.

It would seem likely fromthis result that the condensation product was more stable in alkaline than in acid solution.

The remainder of the methyl alcoholic solution of chitose (160 ml.) containing 0.05% acetic acid was shaken

The solid was again taken up in 100 ml. of dry methyl alcohol containing 0.1% acetic acid, left at room temperature for 24 hours then as before made alkaline to moist litmus paper and evaporated to a dry solid.

Methoxyl content of the product ... ... 12.5%.

with 0.2 g. anhydrous sodium carbonate making it distinctly alkaline to litmus. The solution was evaporated at low temperature *in vacuo* to a viscous syrup then at 30-35°C. to a dry solid. This was dissolved and made up to 160 ml. in dry methyl alcohol to which was added 0.16 g. of glacial acetic acid. The resulting solution reduced Fehling's solution in the cold but the extent of reduction was considerably decreased after it had been standing at room temperature for 3 hours. After standing at room temperature for 24 hours the solution was made alkaline by shaking with 0.2 g. of anhydrous sodium carbonate and evaporated *in vacuo* to a dry solid mass.

Methoxyl content of the product .... 12.7%.

The material was still almost completely insoluble in acetone, ethyl acetate and isopropyl alcohol which indicated that it contained practically no chitose dimethylacetal so that the condensation product was probably methyl chitoside. The fact that no increase in methoxyl content resulted from the third treatment of the material with acidified dry methyl alcohol indicated that the chitose condensation was practically complete after the second treatment.

Some hydrolysis was suspected even in the evaporation of the alkaline solutions for the extent of Fehling's reduction increased when the methyl alcoholic chitose solution was made alkaline, evaporated to a dry solid and taken up again in the same volume of methyl alcohol.

Methylation of methyl chitoside syrup.

(1) By Haworth's method.

2.0 g. of the product having a methoxyl content of 12.5% was dissolved in 3 ml. of 30% sodium hydroxide and the yellow mobile syrup thus obtained transferred to a methylating flask immersed in a waterbath at 60°C. The contents of the flask were vigorously stirred during the methylation which was effected with 10 ml. of dimethyl sulphate and 30 ml. of 30% sodium hydroxide added dropwise, simultaneously and at an equivalent rate. The addition of the reagents was completed after 2 hours and the dark red coloured contents of the flask maintained at 60°C. for a further period of 1 hour. After cooling the product was extracted 3 times with 10 ml. volumes of chloroform and the extract dried over anhydrous sodium sulphate, filtered and evaporated in vacuo at 40°C. Thus was obtained only a very small quantity of a dark syrupy product which gave no definite fraction on distillation in high vacuum.

The experiment was twice repeated, the methylations being carried out at 40°C. and 50°C. respectively but in neither case on distillation of the small amount of product was any appreciable constant boiling fraction obtained.

(2) By the Purdie method.

10 g. of methyl chitoside syrup prepared as previously described and having a methoxyl content of 12.2% were

dissolved in a few ml. of dry methyl alcohol. 17.5 g. of methyl iodide were added to the mobile syrup but only a fraction dissolved. Further additions of methyl alcohol were accordingly made until, on shaking, a homogeneous solution was obtained. About 12 ml. of methyl alcohol were required to bring the methyl iodide completely into solution. To this was added 15 g. of dry silver oxide the methylating flask being then attached to a reflux condenser and immersed in a waterbath at 39-40°C. at which temperature it was left for 6 hours. The reaction mixture was filtered and the residue washed with a little methyl alcohol. The clear filtrate which was alkaline in reaction was evaporated in vacuo at 30°C. to a viscous syrup.

The methylation process was repeated though in this case it was necessary to add only 8 ml. of methyl alcohol to bring the 17.5 g. of methyl iodide into solution. The greater part of the product thus obtained was found to be soluble in acetone and this in 10 ml. of acetone was again methylated with 17.5 g. of methyl iodide and 15 g. of dry silver oxide. The product obtained from the third methylatio was 4.2 g. of a moderately mobile yellow syrup which was again methylated without the addition of acetone as it dissolved in methyl iodide quite readily. From the fifth and final methylation was obtained 3.8 g. of a fairly mobile syrup which was distilled in high vacuum. The first and only constant boiling fraction came over as a mobile colourle

liquid at 100-105°C./0.1 mm. Weight of this fraction obtained was 2.1 g.

Methoxyl content .... 53.3%.

Rotation ... $(\alpha)_D^{20^\circ}$ = +46.4° in methyl alcohol.
c = 5.25%.

The substance was soluble in water. It did not reduce Fehling's solution. After hydrolysis by heating in a boiling waterbath for 10 minutes with 1% hydrochloric acid solution and neutralising with sodium carbonate the solution still did not reduce Fehling's solution. The product reacted towards alkali as the ester of an organic acid.

Equivalent weight by hydrolysis and titration with alkali = 295.

The free acid was prepared by adding an excess (6 ml.) of N/1 caustic soda to 1.5 g. of the syrup and heating the solution under reflux on a boiling waterbath for 30 minutes. After cooling 6 ml. of N/1 hydrochloric acid were added and the solution evaporated in vacuo to dryness. The mass was extracted with 10 ml. of dry acetone, filtered and the clear filtrate evaporated in vacuo at 40°C. to a moderately mobile syrup which was distilled under high vacuum. Thus was obtained 0.9 g. of a colourless moderately mobile syrup distilling at 125-130°C./0.00 mm. No other fraction of definite boiling point was obtained in the distillation.

The product was readily soluble in water, giving a solution which was acid to congo red paper.

Equivalent weight of the acid by titration with N/10 alkali = 259.

Methoxyl content .... 44.3%.

From the properties of the acid and its methyl ester it was thought to be an impure sample of trimethyl chitonic acid which could conceivably arise in the Purdie methylation of methyl chitoside by hydrolysis to free chitose and subsequent oxidation and methylation. The following attempts were therefore made to prepare a pure crystalline derivative of the acid from which it could be identified with certainty.

(1) Sodium salt.

A solution of the free acid, just neutralised with N/1 caustic soda solution was allowed to evaporate spontaneously in air at room temperature. The product was obtained as a hard amorphous glassy solid.

(2) Calcium salt.

0.03 g. of the acid dissolved in 1 ml. of water was left in contact with a slight excess of powdered calcium carbonate and the resulting neutral solution filtered and allowed to evaporate spontaneously in air at room temperatur Only an amorphous product resembling the sodium salt was obtained.

(3) Phenylhydrazide.

To 0.03 g. of the acid dissolved in 1 ml. of water was added 0.012 g. (1 mol.) of phenylhydrazine. The solution was allowed to evaporate slowly at room temperature and during the process a red oily product separated from solution. Attempts at crystallising the material from water, methyl alcohol and ethyl alcohol were unsuccessful.

(4) p-Tolylhydrazide.

To 0.03 g. of the acid neutralised by the addition of N/10 caustic soda solution was added 0.16 g. (1 mol.) of p-tolylhydrazine hydrochloride. A turbidity rapidly developed in the solution and a red oil separated. Again it was not found possible to crystallise the product.

(5) p-Nitrophenylhydrazide.

0.03 g. of the acid in 1 ml. of water was shaken for 2 hours with 0.018 g. of powdered p-nitrophenylhydrazine but the base did not readily dissolve. On heating a clear orange red solution was obtained which was allowed to cool slowly. A red coloured unerystallisable oil was also obtained in this case.

(6) Semicarbazide.

A solution of 0.03 g. of the acid, neutralised with N/10 caustic soda was shaken with 0.013 g. of semicarbazide

hydrochloride. The resulting clear, almost colourless solution was allowed to evaporate spontaneously in air but only the characteristic crystals of sodium chloride separated from the syrupy product.

(7) Anilide.

1 molecular proportion (0.011 g.) of aniline was added to 0.03 g. of the acid in 1 ml. of water and the mixture shaken vigorously. An almost clear solution was thus obtained. This was filtered and allowed to evaporate in air at room temperature. Attempts to crystallise the oily product were unsuccessful.

(8) Silver salt.

A solution of 0.03 g. of the acid in 2 ml. of water was shaken with a little powdered silver carbonate until the mixture was no longer acid to litmus paper. The clear filtered solution was allowed to evaporate to dryness at room temperature in the dark. On examination of the resulti white solid under the microscope it was seen to be made up of small crystalline needles. The material was extremely soluble in cold water but was recrystallised by cooling its hot solution in methyl alcohol.

The remaining quantity of acid (0.6 g.) was similarly converted to the silver salt by neutralisation of its aqueous solution with excess silver carbonate, filtration

and evaporation of the clear solution in vacuo at 40°C. The white solid mass was recrystallised by dissolving in 15-20 ml of hot methyl alcohol, filtering rapidly through a layer of charcoal and allowing the yellow coloured solution to cool slowly. By filtering and washing with a little cold methyl alcohol the material was obtained as a snow white solid consisting of fine crystalline needles. It was left in a vacuum desiccator in the dark to dry. The weight of the recrystallised product obtained was 0.40 g.

A second purification was attempted by again dissolving in 15-20 ml. of hot methyl alcohol. The material however in this case dissolved only with difficulty and even from solution in 30 ml. of hot methyl alcohol it rapidly separated on slight cooling in microscopic crystals making rapid filtration almost impossible. (The superior solubility of the crude product was probably due to the presence of a considerable quantity of impurity). The material was then dissolved in 50 ml. of hot methyl alcohol and the solution rapidly filtered with suction through a layer of charcoal. The filtrate, which was practically colourless, deposited the white crystalline solid on cooling. This was separated as described above and dried in the dark in a vacuum desiccator over concentrated sulphuric acid. Weight of solid obtained was 0.31 g. A further crop of crystals was obtained by working up the mother liquors.

The pure salt slowly darkened on exposure to light.

Analysis --	C	H	Ag	$.OCH_3$
found.	33.00%	4.98%	33.33%	28.3%
Silver trimethyl chitonate requires ($C_9H_{15}O_6Ag$)	33.03%	4.59%	33.03%	28.4%

Rotation -- $(\alpha)_D^{20^\circ} = +43.30^\circ$ in water. c = 1.570%.

Preparation of the silver salt of trimethyl chitonic acid.

3.5 g. of trimethyl chitonic acid prepared from calcium chitonate as previously described was dissolved in 50 ml. of water and the solution left in contact with an excess (5 g.) of silver carbonate until it was no longer acid to litmus paper. The mixture was filtered and the clear yellow solutio decolourised with a little charcoal. Filtration resulted in an almost colourless solution which was evaporated *in vacuo* at room temperature in a desiccator over concentrated sulphuric acid. Thus was isolated 4.8 g. of a crystalline solid which was slightly discoloured with impurities. It was purified by twice recrystallising from methyl alcohol. The crystalline solid on examination under the microscope appeared identical with that obtained in the preceding experiment.

Analysis —	C	H	Ag	$.OCH_3$
Found.	33.51%	5.28%	33.18%	28.1%
Silver trimethyl chitonate requires ($C_9H_{15}O_6Ag$)	33.03%	4.59%	33.03%	28.4%

Rotation — $(\alpha)_D^{20^\circ}$ = +43.24° in water. c = 4.440%.

Action on chitose of dimethyl sulphate and 30% caustic soda solution in an acid medium.

30 ml. of chitose solution estimated to contain approximately 2 g. of chitose were evaporated in vacuo at 20-25°C. to a mobile syrup about 3 ml. in volume. On addition of 3 ml. of dimethyl sulphate to this syrup and shaking the mixture vigorously a milky emulsified solution was obtained which was distinctly acid to litmus paper. 2 drops of phenolphthalein indicator were added to the mixture and the reaction flask was surrounded by a waterbath at 30°C. The contents of the flask were vigorously stirred during a slow dropwise addition of 30% caustic soda solution. After 1 ml. of alkali had been added further addition caused the development of the red colour of phenolphthalein and at the same time the colloidal nature of the mixture was destroyed by its separation into 2 distinct phases. A further addition

of alkali caused a darkening of the reaction mixture indicating that decomposition had occurred. The restoration of the colloidal state and the acid reaction of the mixture was effected by adding a further 3 ml. of dimethyl sulphate. A further 1 ml. of 30% alkali was then added before the mixture again became alkaline and separated into 2 distinct phases. At this point the mixture was cooled and several more drops of alkali were introduced to ensure that the aqueous phase remained alkaline. The addition of 5 ml. of water precipitated practically all the excess dimethyl sulphate from the aqueous layer which was removed in a separating funnel. To make certain that no acidity developed in the solution 2 g. of powdered calcium carbonate were added and the mixture was evaporated *in vacuo* at 30°C. to a dry solid mass. An attempt to extract any methyl chitoside from the product with cold dry methyl alcohol resulted in the dissolution of a considerable amount of the inorganic material Most of the carbohydrate content of the mixture was finally separated by extraction with cold absolute ethyl alcohol which dissolved only a small amount of the inorganic material. By filtration and evaporation of the clear solution *in vacuo* at 30°C. 1.4 g. of a solid amorphous product was obtained.

Methoxyl content of the product ... 12.3%.

The solid did not reduce cold Fehling's solution nearly so strongly as did the same weight of amorphous chitose.

The above experiment was repeated using twice the quantities of dimethyl sulphate (12 ml.) and 30% caustic soda (4 ml.) in the methylation process.

Methoxyl content of the product .... 12.7%.

Methylation of "methyl chitoside" syrup prepared by the action of 30% caustic soda on a mixture of chitose and dimethyl sulphate.

(1) By Haworth's method.

0.8 g. of the solid material dissolved in 1 ml. of water was transferred to a small flask and methylated at 45-50°C. in the usual manner with 2.5 ml. of dimethyl sulphate and 7.5 ml. of 30% caustic soda solution. The mixture darkened considerably during the process and only a very small quantity of a chloroform soluble product was obtained which yielded no appreciable fraction of constant boiling point on distillation in high vacuum.

(2) By Purdie's method.

2 g. of the substance were methylated with 3.5 ml. of methyl iodide (just sufficient methyl alcohol to form a homogeneous solution was also added) and 3 g. of silver oxide at 35-40°C. After 5 hours at this temperature the

the reaction mixture was heated to 60°C. for a further period of 1 hour. After 4 similar methylations the product was extracted with acetone. Evaporation of the clear extract in vacuo at 40°C. to constant weight gave 0.85 g. of a moderately mobile syrup which was twice methylated with 3.5 ml. of methyl iodide and 3 g. of silver oxide. Distillation of the final product produced several drops of a colourless mobile liquid boiling at 100 - 105°C./0.1 mm. This product ha no reducing action on Fehling's solution either before or after hydrolysis but behaved towards alkali as the ester of an organic acid. The silver salt prepared as previously described was obtained in a crystalline form identical with that of trimethyl chitonic acid though not in sufficient quantity for analysis.

Preparation of chitose dimethylacetal by the action of dry methyl alcoholic hydrochloric acid solution on chitose.

A quantity of almost dry amorphous chitose prepared from 40 g. of glucosamine hydrochloride and estimated to contain approximately 25 g. of chitose was taken up in 30 ml. of cold dry methyl alcohol and the solution filtered from the insoluble salts. The clear yellow filtrate was then made up to 400 ml. with dry methyl alcohol and to the

resulting pale yellow liquid which contained some sodium acetate was gradually added a solution of 10% hydrochloric acid in dry methyl alcohol until a test portion imparted a blue colour to congo red paper. A further 10 ml. of the methyl alcoholic hydrochloric acid solution was introduced so that the final solution contained approximately 0.25% hydrochloric acid. The rotational changes of the solution at room temperature, followed over a period of 7 days are recorded in the following table.

Time after acidification of the solution.	Observed rotation at room temperature in a 1 dm. tube.
10 mts.	+2.59°
3 hrs 30 mts.	+2.56°
7 "	+2.53°
24 "	+2.49°
48 "	+2.47°
72 "	+2.45°
96 "	+2.44°
120 "	+2.43°
144 "	+2.44°
168 "	+2.43°

The solution was made alkaline to moist litmus by shaking with successive small quantities of anhydrous sodium carbonate and evaporated *in vacuo* at 30°C. to a stiff syrup.

Extraction of the product with three 100 ml. volumes

of ethyl acetate and evaporation of the combined extracts *in vacuo* at 30°C. yielded 9.1 g. of a viscous yellow syrup.

Expecting that by a second treatment of the ethyl acetate insoluble residue with methyl alcoholic hydrochloric acid a further small quantity of the ethyl acetate soluble product would be obtained it was taken up in 200 ml. of dry methyl alcohol containing 0.5% hydrochloric acid and the solution left for 7 days at room temperature. A surprisingly large yield amounting to 12.0 g. of the ethyl acetate soluble material was obtained on treatment of the solution as described above.

It appeared from this result that the fall in rotation observed during the first treatment with methyl alcoholic hydrochloric acid gave no indication of the rate or extent of condensation of chitose and methyl alcohol but was probably due to the action of the reagent on a chitose decomposition product. It seemed likely that the smaller yield obtained in the first case was due to the fact that the condensation process which proceeded without effecting an appreciable change in rotation of the solution was interrupted before the state of equilibrium had been attained. The higher concentration of hydrochloric acid used in the second case would then account for the larger amount of condensation product due to its acceleration of the reaction rate.

The combined ethyl acetate soluble extracts from these two condensation experiments contained a quantity of an ether

soluble material which was removed by repeated extraction with 10 ml. volumes of dry ether. By evaporation of the combined ether extracts 1.3 g. of a light yellow viscous syrup was obtained.

The remaining ethyl acetate soluble - ether insoluble fraction was dissolved in 350 ml. of ethyl acetate and the solution to which was added a little charcoal was dried by leaving in contact with anhydrous sodium sulphate for 24 hours. Filtration gave a clear yellow liquid which was evaporated <u>in vacuo</u> at 30°C. to a viscous syrup. Traces of remaining solvent were then removed under high vacuum at 40-45°C.

Yield of ethyl acetate soluble fraction ... 19.8 g.

Analysis —	C	H	$.OCH_3$
found ..	47.16%	7.92%	28.7%
Chitose dimethyl-acetal ($C_8H_{16}O_6$) requires	46.15%	7.69%	29.8%

Rotation — $(\alpha)_D^{15^\circ} = +30.95^\circ$ in water. c = 9.467%.

The rotation in neutral aqueous solution remained unchanged over a period of 24 hours.

METHYLATED DERIVATIVES FROM CHITOSE DIMETHYLACETAL.

Preparation of trimethyl chitose dimethylacetal.

10 g. of the chitose dimethylacetal prepared in the preceding experiment were dissolved in 5 ml. of water and the resulting mobile liquid methylated with 30 ml. of dimethyl sulphate and 90 ml. of 30% caustic soda solution. The reaction was carried out initially at 45°C. with slow simultaneous addition of equivalent quantities of the reagents After about ⅓ of the methylating reagents had been added the reaction mixture was heated to 60°C., at which temperature the additions of alkali and dimethyl sulphate were completed. The reaction mixture was then heated to 100°C. for half an hour, cooled and extracted three times with 50 ml. volumes of chloroform. The combined extract, after drying over anhydrous sodium sulphate, was evaporated *in vacuo* at 35-40°C. to a mobile syrup. Distillation of the product in high vacuum yielded 6.1 g. of a colourless mobile liquid which came over at 90-95°C./0.08 mm.

Methoxyl content of the product ... 60.1%.

Trimethyl chitose dimethylacetal requires ... 62.0%.

The product, after further methylation by the method of Purdie, was redistilled in high vacuum and thus was obtained 6.0 g. of a colourless liquid distilling at 90-92°C./ 0.08 mm.

Analysis —	C	H	.OCH$_3$
found ..	53.00%	8.73%	61.1%
Trimethylchitose dimethylacetal ($C_{11}H_{22}O_6$) requires	52.80%	8.80%	61.0%

Rotation — $(\alpha)_D^{15^\circ}$ = +39.77° in water. c = 8.197%.

The rotation of the solution was unaltered after 7 days.

Hydrolysis of trimethylchitose dimethylacetal.

To 0.8 g. of trimethylchitose dimethylacetal in 10 ml. of a neutral aqueous solution was added 0.3 ml. of concentrated hydrochloric acid giving the solution an approximate concentration of 1% hydrochloric acid. The rotation of the solution decreased slightly over a period of 3 days (from +3.22° to +3.12°), after which it remained constant over a further period of 4 days. To a test portion of the liquid was added just sufficient sodium carbonate to neutralise the acid present and to this was added an equal volume of Schiff's reagent. Only a very faint pink colour slowly developed in this solution. To 1 ml. of the acid solution neutralised with a little sodium carbonate was added 0.05 g. of semicarbazide hydrochloride and 0.07 g. of sodium acetate. None of the characteristic crystals of trimethyl aldehydochitose semicarbazone separated when this was allowed to

evaporate spontaneously at room temperature. The tests indicated that under these conditions the hydrolysis of trimethylchitose dimethylacetal had proceeded to only a negligible extent. The hydrochloric acid in the original solution was then made up to a strength of approximately 5% and the liquid again left at room temperature. No appreciable change was observed in the rotation of this solution over a period of 7 days. At the end of this period, however, a neutralised portion of the solution rapidly restored the colour to Schiff's reagent. 5 ml. of the solution was neutralised with silver carbonate and to the filtered liquid was added 0.25 g. of semicarbazide hydrochloride and 0.31 g. of sodium acetate. On allowing the solution to evaporate spontaneously at room temperature to small volume a comparatively large amount of crystalline material separated. The product was recrystallised from dry methyl alcohol.

Weight of purified material ... ... 0.25 g.

Melting point ... ... 148°C.

Mixed melting point with trimethyl aldehydochitose semicarbazone. ... 148°C.

The yield of the product thus obtained was 60% of the theoretical amount.

ATTEMPTED DEGRADATION OF METHYLATED CHITOSE DERIVATIVES.

Hydrolysis and oxidation of trimethylchitose dimethylacetal with nitric acid.

15 g. of trimethylchitose dimethylacetal were dissolved in 150 ml. of cold nitric acid (d 1.42) and the solution was gradually heated on a water bath. At a bath temperature of 35-40°C. a strong reaction set in whereupon the reaction flask was cooled under cold running water. When the vigour of the reaction had subsided the flask was again slowly heated in the waterbath to 90°C. at which temperature it was left for 15 minutes. The reaction at this point appeared to have almost ceased and the solution after diluting with 100 ml. of water was evaporated *in vacuo* to a syrup which was repeatedly taken up in an equal volume of water and the solution evaporated *in vacuo* to remove as much nitric acid as possible. The product was finally dried *in vacuo* at 100°C. for 1 hour. Distillation of the resulting syrup in high vacuum gave 10.2 g. of a moderately mobile syrup boiling at 125-130°C./0.08 mm. and no other fraction distilling at a constant temperature was obtained.

The product was dissolved in 100 ml. of water and the solution shaken with 15 g. of powdered silver carbonate until it was neutral to litmus paper. The excess of silver carbonate was removed by filtration and the clear solution evaporated *in vacuo* at 40°C. until a quantity of white solid

had crystallised out. By cooling in the refrigerator and rapidly filtering a considerable quantity of solid material was obtained. On examination under the microscope it was seen to consist of fine crystalline needles identical in form with the characteristic crystals of silver trimethyl chitonate. A further yield of the compound was obtained by working up the mother liquor. Total weight of the crude air dried silver salt obtained was 10.1 g. The pure compound was prepared by recrystallising from dry methyl alcohol and drying in the dark in a vacuum desiccator over concentrated sulphuric acid.

Analysis —	C	H	Ag	$.OCH_3$
found ..	33.06%	4.40%	33.37%	28.2%
Silver trimethyl chitonate ($C_9H_{15}O_6Ag$) requires ...	33.03%	4.59%	33.03%	28.2%

Rotation — $(\alpha)_D^{15^\circ} = +43.18^\circ$ in water. c = 5.187%.

The oxidation product was thus identified as trimethyl chitonic acid.

The effect of prolonged treatment of trimethyl chitonic acid with nitric acid.

A solution of 2 g. of trimethyl chitonic acid in 25 ml. of nitric acid (d 1.42) was heated in a waterbath at 90°C. for 6 hours. The solution was ~~evaporated~~ diluted with an equal volume of water and evaporated *in vacuo* at 45°C. to a syrup. The product was repeatedly taken up in water and evaporated at 45°C. *in vacuo* to remove nitric acid and finally heated *in vacuo* to 100°C. for 30 minutes to get rid of the last traces of water. By distillation in high vacuum 1.7 g. of a colourless, moderately mobile syrup boiling at 125-127°C./0.08 mm. was obtained.

Equivalent weight of the product 193.2

Theoretical value for trimethyl chitonic acid .. 220.

Rotation — $(\alpha)_D^{20^\circ}$ = +64.69°. in water. c = 4.436%.

Rotation of trimethyl chitonic acid +69.89°C.

Suspecting from these figures that the syrup was essentially trimethyl chitonic acid containing some impurities, the preparation of the silver salt was undertaken as previously described. The crude solid product obtained separated on recrystallising from dry methyl alcohol in crystalline needles having the characteristic form of silver trimethyl chitonate.

Rotation — $(\alpha)_D^{20^\circ}$ = +43.20°%. in water. c = 4.620%.

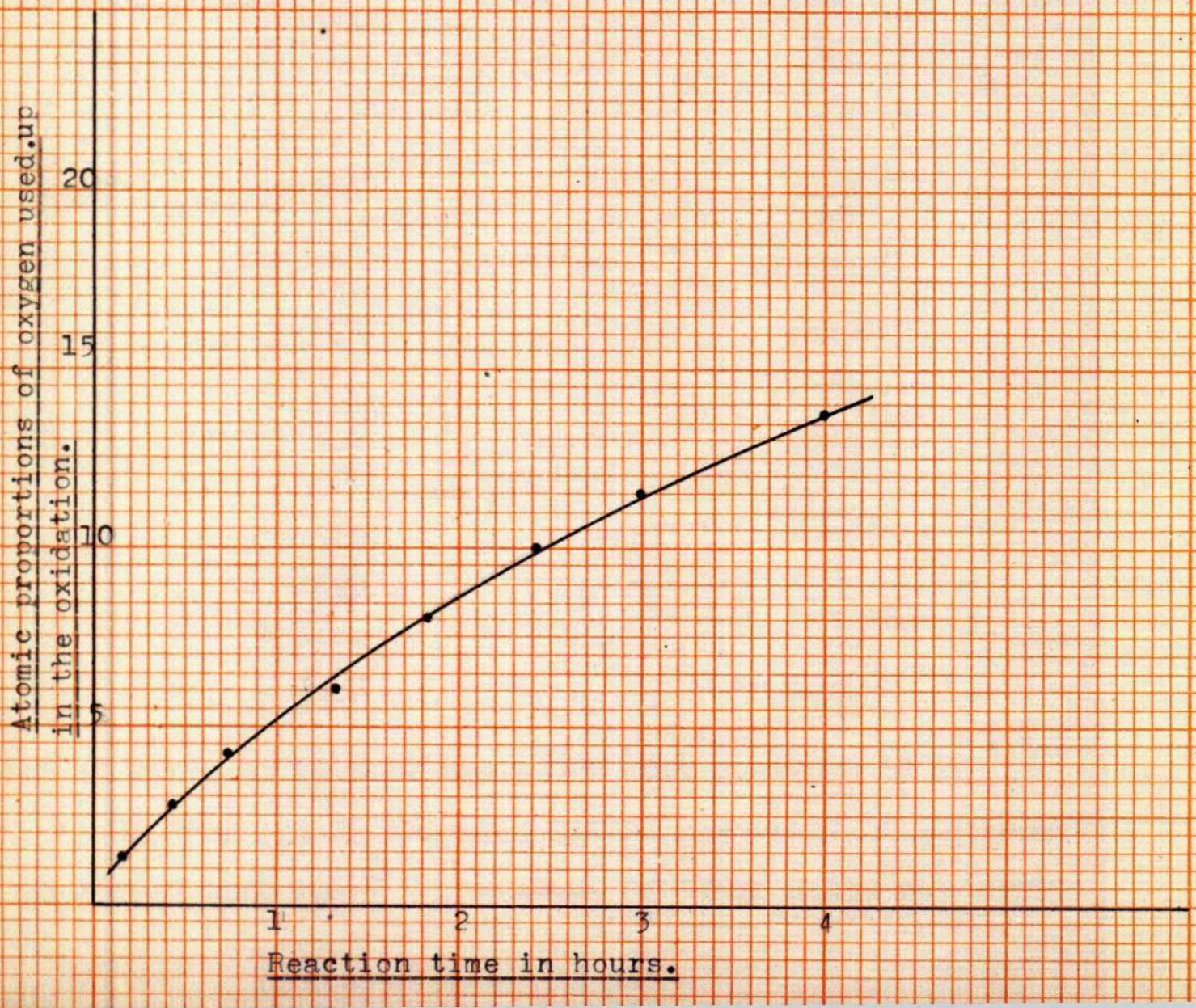
Rotation of silver trimethyl chitonate ... $(\alpha)_D^{20^\circ}$ = +43.24°%. in water. c = 4.440%.

Oxidation of trimethyl chitonic acid with alkaline permanganate.

0.0601 g. of trimethyl chitonic acid were dissolved in 100 ml. of N/2 sodium hydroxide solution containing 0.3440 g. of potassium permanganate (equivalent to 20 atomic proportions of oxygen). 5 ml. of the freshly prepared solution, acidified with an excess of 20% sulphuric acid solution and titrated with a standard solution of ferrous ammonium sulphate in 10% sulphuric acid, was found to be equivalent to 4.05 ml. of the latter. The solution was allowed to remain at room temperature, 5 ml. being removed at noted intervals of time, acidified and titrated with the ferrous ammonium sulphate solution. The results, indicated in the following table, showed that the rate of oxidation of trimethyl chitonic acid with alkaline permanganate at room temperature was negligible.

GRAPH 8.

The oxidation of trimethyl chitonic acid with alkaline permanganate.



Reaction time at room temperature.	Titration with ferrous ammonium sulphate.
2 mts.	4.05 ml.
10 "	4.05 ml.
20 "	4.05 ml.
45 "	4.05 ml.
60 "	4.05 ml.

The reaction flask was then immersed in a waterbath at 70°C. and again the rate of oxidation followed by back titration of the remaining permanganate with standard ferrous ammonium sulphate solution. The results are indicated in the following table and greph 8.

Reaction time at 70°C.	Titration with ferrous ammonium sulphate.
10 mts.	3.75 ml.
25 "	3.45 ml.
45 "	3.15 "
1 hour 20 mts.	2.80 "
1 " 50 "	2.40 "
2 " 25 "	2.00 "
3 " 0 "	1.70 "
4 " 0 "	1.25 "

It is apparent from the greph that at no stage in the reaction is there any marked decrease in the rate of oxidation which would be expected if there were formed an

intermediate product stable to the action of alkaline permanganate.

Attempted oxidation of trimethyl chitonic acid with Fenton's reagent.

Following the directions of Neuberg, Wolff and Niemann (Ber. 35, 4009) who claimed to have obtained a pentose from chitaric acid by this method, a solution of calcium trimethyl chitonate (1.05 g.) in 8 ml. of water in which was dissolved 1 g. of ferrous sulphate was treated at room temperature with 1 ml. of 100 volume hydrogen peroxide solution (1 molecular proportion) added dropwise with stirring. Since in this case there was no sign of gas evolution as was observed by the above workers during their oxidation of calcium chitarate it was suspected that no degradation had taken place. A further 1 ml. of hydrogen peroxide was added and the mixture left at room temperature overnight. It was then filtered and the clear solution evaporated *in vacuo* at 40°C. to dryness. A solution of the residue in 10 ml. of 20% sulphuric acid was extracted 3 times with 5 ml. volumes of chloroform and the combined chloroform extract freed from traces of sulphuric acid by shaking with a little powdered barium chloride. The mixture was filtered and the solution dried by leaving in contact with anhydrous sodium sulphate overnight. The solutio

was again filtered and evaporated in vacuo at 40°C. to a syrup. Distillation of the product gave 0.53 g. of a colourless moderately mobile liquid boiling at 125-127°C./ 0.08 mm.

Rotation ... $(\alpha)_D^{20^\circ}$ = +68.24° in water. c = 3.121%.

The fact that the product was unchanged trimethyl chitonic acid was confirmed by neutralising its aqueous solution with silver carbonate and isolating as previously described the characteristic crystals of silver trimethyl chitonate.

Attempted degradation of trimethyl chitonic acid by the Hoffmann reaction.

Preparation of trimethyl chitonamide.

A quantity of the amide of trimethyl chitonic acid was prepared as described in a previous experiment. In this case, however, the syrup obtained by evaporation in vacuo of the trimethyl chitonic acid solution in methyl alcohol saturated with ammonia had crystallised after leaving in the refrigerator overnight. Purified by recrystallising from dry acetone it was obtained as a mass of white crystalline needles.

Yield from 6 g. of trimethyl chitonic acid was 4.6 g.

Melting point. ... ... 115-116°C.

Rotation .. $(\alpha)_D^{20^\circ}$ = +47.60°ψ. in water. c = 2.311%.

The rotation of the solution had not altered after 48 hours at room temperature.

The trimethyl chitonamide syrup prepared 6 years previously rapidly crystallised when seeded with a few crystals of this product.

Hoffmann degradation of trimethyl chitonamide.

An alkaline hypobromite solution was prepared by dissolving 2.4 g. of sodium hydroxide in 20 ml. of distilled water, cooling the solution to -5°C. in a freezing mixture and shaking at this temperature with 0.6 ml. of bromine.

To 14.7 ml. of the freshly prepared hypobromite solution at -5°C. was gradually added 1.5 g. of powdered trimethyl chitonamide the mixture being vigorously shaken during the addition. The solid amide dissolved almost immediately and the solution became slightly milky with the separation of an oily product. Shaking at this temperature was continued for 15 minutes but the turbidity was not thereby removed. When the mixture was allowed to stand for a few minuten the oily product collected at the bottom of the reaction flask. This product which was only very small in amount, was considered to be a reaction byproduct.

The reaction flask was then immersed in a waterbath at 75°C. whereupon a strong ammoniacal odour was detected in the evolved vapours which turned moist litmus paper blue. A yellow colouration rapidly developed in the solution but this did not increase in intensity after the first minute of the heating period. The solution was maintained at this temperature for 20 minutes and then cooled to 0°C. Extractio of the liquid with three 15 ml. volumes of ether and evaporation of the combined extract *in vacuo* at 30°C. resulted in the isolation of only a minute amount of a dark syrup. The aqueous solution which still contained the bulk of the product was acidified with an excess of hydrochloric acid and again extracted with ether. 1.0 g. of a moderately mobile syrup was obtained by evaporation of this extract at 25-30°C. *in vacuo*. Distillation of the product in high vacuum gave 0.85 g. of a colourless syrup which came over at 125-127°C./0.08 mm. It reacted towards alkali as an acid.

Equivalent weight of the product .... 207.

Rotation .. $(\alpha)_D^{20^\circ}$ = +66.08°C. in water. c = 3.80%.

The suspicion from these figures that the product was trimethyl chitonic acid was confirmed by the preparation of the crystalline silver salt in the usual way.

Rotation of silver salt .. $(\alpha)_D^{20^\circ} = +43.16^\circ$ in water.
c = 3.881%.

Rotation of the silver salt
of trimethyl chitonic acid .. $(\alpha)_D^{20^\circ} = +43.24^\circ$ in water.
c = 4.440%.

Attempted preparation of crystalline 3:4:6 trimethyl glucosazone from trimethyl aldehydochitose.

0.9 g. of the syrupy trimethyl aldehydochitose phenylhydrazone prepared as previously described, was dissolved in just sufficient 50% acetic acid solution to keep the material in solution at room temperature and to this was added 0.61 g. (2 mols.) of phenylhydrazine. One half of this solution was heated for 2 hours in a boiling waterbath and the other left at room temperature for 14 days. In both cases an oily product gradually separated from solution but attempts at crystallising this material from a large number of solvents were unsuccessful.

SUMMARY.

(1) The report of Zechmeister and Toth (Ber., 66B, 522) that d-arabinosone is present in solutions of chitose prepared by deamination of glucosamine hydrochloride has been confirmed. Furthermore, it has been demonstrated that this osone is formed during aeration with atmospheric air, the method used to free the final solution from nitrous acid.

(2) Chitose 2:4dinitrophenylhydrazone, chitose p-nitrophenylhydrazone and chitose benzylphenylhydrazone have been isolated as pure crystalline derivatives.

(3) The preparation of d-glucosazone by the action of phenylhydrazine on a solution of chitose formed from pure chitose 2:4dinitrophenylhydrazone has proved that the formation of d-glucosazone from the deamination product of glucosamine hydrochloride is not solely a product of reaction between phenylhydrazine and undeaminated glucosamine hydrochloride, as suggested by Fischer and Andreae (Ber., 36, 2587).

(4) Attempts to prepare the crystalline compounds, tribenzoyl chitose and methyl chitoside described by Neuberg, Wolff and Niemann (Ber., 35, 4009) have been unsuccessful.

(5) Evidence has been produced for the existence of an unstable methyl chitoside formed by the condensation of chitose and methyl alcohol in a faintly acid solution. In a more strongly acid medium (1% HCl) the condensation proceeds further with the formation of the relatively stable chitose dimethylacetal. These condensations with methyl alcohol are not indicated by changes in the rotatory power of the solutions.

(6) Complete methylation of the hydroxyl groups of chitose dimethyl acetal has resulted in the formation of trimethylchitose dimethylacetal which, on hydrolysis, yields trimethyl aldehydochitose. These compounds have been isolated as syrups only but the semicarbazone of trimethyl aldehydochitose has been prepared in pure crystalline form.

(7) The non glucosidic structure and aldehydic nature of trimethyl aldehydochitose has been confirmed from its preparation by complete methylation and subsequent hydrolysis of chitose diethylmercaptal, the method usually employed in the preparation of aldehydo-sugar derivatives.

(8) Deamination of 3:4:6trimethyl glucosamine hydrochloride did not result in the expected formation of trimethyl aldehydochitose. The process was accompanied by a partial demethylation and the product was identified

as ω-methoxy-5-methyl furfural.

(9) Oxidation of trimethyl aldehydochitose with concentrated nitric acid gave rise to trimethyl chitonic acid, the same compound having been prepared by methylation of chitonic acid. Trimethyl chitonic acid, a semi-mobile syrup, has been characterised by the preparation of two crystalline derivativ the silver salt and acid amide.

(10) It has not been found possible to estimate the chitose content of a solution of deaminated glucosamine hydrochloride by the usual methods of sugar estimation owing probably to the presence of interfering decomposition product A gravimetric estimation method has been devised based on the rapid condensation of chitose and p-nitrophenylhydrazine with the formation of the insoluble crystalline chitose p-nitrophenylhydrazone. The presence of glucosamine hydrochloride, glucose or mannose does not interfere with this chitose estimation method.

(11) A study of the extent of deamination of glucosamine hydrochloride under varying conditions and of the stability of chitose has led to the preparation of a deaminat glucosamine hydrochloride solution containing 93.7% of the theoretical quantity of chitose as compared with the 64.5% yield obtained by the method of Zechmeister and Toth

(Ber., 66B, 522).

(12) Further evidence has been sought for the existence of the chitose anhydro ring in the 2:5 position in the attempted degradation of trimethyl chitonic acid to methylat products of known constitution. Owing to the unusual stabil of this compound, however, attempts at oxidation with concentrated nitric acid and Fenton's reagent and degradation by the Hoffmann reaction were unsuccessful, only unchanged trimethyl chitonic acid being recovered from the reaction product in each case.

The results of these studies have confirmed the hypothesis of Levene and La Forge (J. Biol. Chem., 20, 453) that chitose is 2:5 anhydromannose.

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